Second Edition

Physiological Systems in Insects

Marc J. Klowden

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To my wonderful children, Dan and Amanda

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Marc J. Klowden

University of Idaho
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Preface

The number of insects on our planet and their incredible diversity of structure and function are mind-boggling. Insects live anywhere and everywhere. To a non-entomologist, most insects are indistinguishable from one another and simply look the way insects are supposed to look. Head, thorax, abdomen, six legs, and often wings, give little insight into what has contributed to the incredible success of these tiny animals. However, to one trained in the subtleties of six-legged science, the physiological systems responsible for their varied lifestyles explain it all. Every ecological peculiarity of insects has a physiological basis, and to understand their distribution and ecological roles in varied environments, one must understand the systems that allow them to function so well.

A good example is the mosquito, *Anopheles gambiae*, a major vector of malaria and responsible for millions of human deaths each year. Its designation as the "world's most dangerous animal" is well deserved but oddly, not shared with several other species that look identical even under the microscope. It turns out that *Anopheles gambiae* is but one member in a species complex that consists of several other mosquito species that are morphologically indistinguishable, but physiologically and behaviorally incapable of transmitting the agents of malaria. *Aedes aegypti*, the yellow fever mosquito, has all the basic mosquito features of *Anopheles gambiae*, and is an important vector of dengue and yellow fever, but it can't transmit malaria to humans on a bet. Relatively minor physiological differences can have enormous implications.

To begin to describe all these physiological peculiarities, an ideal treatise on insect physiology would consist of over a million volumes, one for each of the known species, with each volume containing a detailed description of the physiological systems that make up the whole insect. This would first require a substantial increase in the office space allotted to insect physiologists. For the time being, we have to compromise and acknowledge both the constraints of space and the extent of our knowledge.

The constraints on our knowledge are undoubtedly the most substantial. An embarrassingly few of those species we know about have been examined in any depth. Those of us who study insects often choose our subjects based on their perceived importance to society and ease of rearing, with an emphasis on the ease of rearing. Developing a system to rear and study a finicky and uncommon species is a major project in itself, and funding agencies increasingly tend not to favor proposals that will study any of the more than 90% of insects that never cross paths with humans. Insects have been recognized as superb models for studying evolution, genetics, and physiology, and are being used more than ever in studies of molecular biology. The genomes of about a dozen insects have been sequenced, with over half of these being species of *Drosophila*. The others are important models for studies of complex behavior, such as the honey bee *Apis mellifera*, and for the transmission of parasites, including *Anopheles gambiae* and *Aedes aegypti*. What has resulted is a fairly comprehensive understanding of a handful of insects, but far from what we could honestly generalize as "insect" physiology. Whenever I go before our insect physiology class on the first day, I always apologize for so much of the course being devoted to *Drosophila*. I must reluctantly offer the same apologies to readers of this book.

This second edition has been significantly updated with recent information on the molecular underpinnings of physiological systems. As in the first edition, I kept the citations to references out of the text to maintain the flow of reading, but have substantially increased the references at the end of each chapter. I hope that readers will use this resource when they are unsatisfied with the limitations of the text discussion. My objective in this edition is once again to walk the line between educating biologists who choose to use insects in their work on biological systems and entomologists trying to better understand the animals they love.

This book is primarily intended to supplement our cooperative insect physiology course at the University of Idaho and Washington State University. Both the course and this book have benefitted enormously from my long and meaningful teaching collaboration with John Brown at Washington State University. I thank John for the very enjoyable and enlightening partnership over the past 20 years that has significantly influenced my directions in teaching and writing.

CHAPTER 1*

Endocrine Systems

Hormones are the chemical messengers of multicellular organisms that allow the cells to communicate and engage in coordinated responses. They are especially pervasive in insect systems, affecting a wide variety of physiological processes including embryogenesis, postembryonic development, behavior, water balance, metabolism, caste determination, polymorphism, mating, reproduction, and diapause. Hormones work along with the nervous system to provide the necessary communication between all the cells that make up a multicellular animal.

There is good reason to have two communications systems existing side by side. The nervous system is certainly capable of sending electrical messages rapidly via nerves, but a network of nerves that reached every cell and coordinated its activities would take an enormous amount of space. In endocrine systems, where chemical messengers are transported in the blood, all tissues can receive the message as long as they have receptors that enable them to recognize it. Hormones thus allow a sustained message to be sent to all cells, but only those cells that possess the receptors are capable of responding. For example, the molting process in many insects requires hours for its full completion. It could occur faster if it were coordinated by the nervous system, but that would mean that

*Bold terms are meant to indicate importance and/or inclusion in the Glossary.

every epidermal cell involved would have to receive a nervous message, hopelessly complicating the internal environment with neurons and leaving little room in the body for other organs. Some cells may not participate in molting; these are oblivious to the hormonal conditions because they lack receptors. Some processes, such as feeding and escape, cannot rely on the slowness of the endocrine system and are regulated by the nervous system. If information regarding some threat in the environment, such as a predator, were to be relayed by the endocrine system to initiate escape behavior, the insect would probably be eaten well before the message arrived. By selecting hormones as a messenger for some systems, insects have made a trade-off between the speed of the response and the complexity of the system that would be required to implement it.

The classical definition of a **hormone**, a word coined from the Greek for "I excite," includes those substances secreted by glands and transported by the circulatory system to other parts of the body, where they evoke physiological responses in target tissues in minute quantities. Although the term "endocrine" originally implied that multicellular glands were the sources of the chemical messengers, it is now recognized that hormones may also be produced by single cells that are not necessarily clustered into a distinct gland. In addition to these more discrete endocrine glands, a number of neurosecretory cells are found throughout the body that also produce hormones.

TYPES OF HORMONE RELEASE SITES IN INSECTS

Insects have classical **endocrine glands**, which are tissues that specialize in the secretion of chemical messengers that are transported by the blood and act on receptor-bearing target tissues elsewhere in the body (Figure 1.1A). Examples of endocrine glands in insects are the **prothoracic glands**, which produce ecdysteroids, and the **corpus allatum**, which produces juvenile hormones. Insects, like vertebrates, also have **nerve cells** that generate electrical impulses and translate these to chemical messages at the synapse and then propagate the messages to other neurons to which they are connected. In this case, the messenger, or **neurotransmitter**, binds to receptors on the postsynaptic neuron, remaining compartmentalized within the synapse and not entering the bloodstream. The neurotransmitter can thus be considered as a hormone that is acting locally within the synapse (Figure 1.1B). The neurotransmitters may also be released directly at an endocrine cell (Figure 1.1C). Insects also have functional hybrids of neurons and endocrine glands called **neurosecretory cells**. Neurosecretory cells are specialized neurons that produce chemical messengers that are released into the bloodstream and affect distant target tissues. Rather than doing this at the synapse between two neurons, the chemicals are released into circulation or delivered to cells at a specialized structure called a **neurohemal organ** (Figure 1.1D).

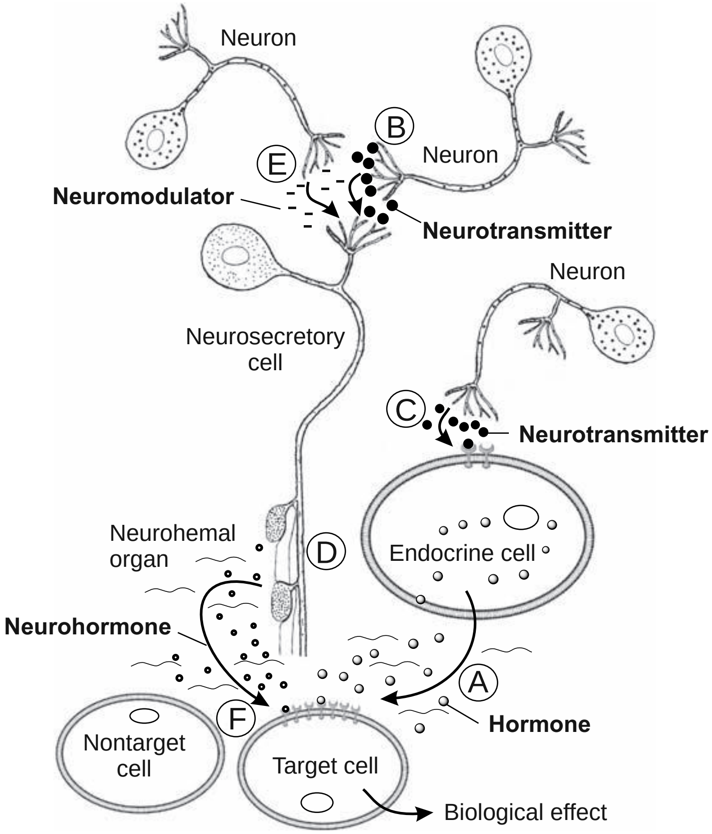


FIGURE 1.1. Some examples of neurotransmitter release. A. An endocrine cell releasing a hormone into the circulatory system. B. A neuron synapsing with a neurosecretory cell, releasing a neurotransmitter at the synapse. C. A neuron synapsing with an endocrine cell, releasing a neurotransmitter. D. A neurosecretory cell releasing a neurohormone into the circulatory system at a neurohemal organ. E. An inhibitory neuron synapsing with a neurosecretory cell, releasing a neuromodulator at the synapse. F. Receptors on target cells recognize specific neurohormones in circulation, resulting in a biological effect. The absence of receptors on nontarget cells results in the cell not being able to respond to the circulating chemical messages, and any molecules taken up nonspecifically are degraded.

Thus, the utilization of chemical messengers lies on a spectrum, with neurons at one end that provide a local release of neurotransmitter that affects other neurons only, neurosecretory cells in the middle with their modified neurons releasing neurohormones into general circulation, and conventional endocrine glands at the other end releasing hormones into general circulation. The chemical products released from these various sites are referred to as hormones if they are

produced by endocrine glands, neurotransmitters if produced by neurons, and **neurohormones** if produced by neurosecretory cells. **Neuromodulators** may be released by neurons at the synapse and modify the conditions under which other nerve impulses are transmitted and received (Figure 1.1E). Receptors present on the postsynaptic membrane and on target cells specifically bind the molecules and produce a biological effect, but nontarget cells that lack these receptors are unable to receive the message.

EARLY EXPERIMENTS THAT SET THE STAGE FOR OUR CURRENT UNDERSTANDING

The first evidence for the existence of hormones in insects is attributed to **Bataillon** in 1894, although at the time, the actual involvement of chemical messengers was not recognized. When silkworm larvae were ligated, separating the anterior and posterior halves with a tightly knotted thread that restricted the flow of hemolymph between the two halves, only the anterior portions of the larvae successfully pupated. However, the result was attributed to differences in internal pressure and not to any hormones.

It was not until the experiments of **Kopeć** in 1917 that the presence of hormones in insects was confirmed. When Kopeć ligated the last instar larva of the gypsy moth just behind the head, the insects pupated normally except, of course, for abnormalities of the head. In contrast, if an earlier instar was ligated in the same way, pupation failed to occur at all. When the ligature was applied to the middle portion of the last instar larva before a critical period had passed, only the anterior half pupated, with the critical period believed to be the time at which hormone was released into circulation from the anterior portion. However, if the ligature was applied after the critical period, both halves pupated (Figure 1.2). Removal of the brain itself before the critical period prevented pupation, but if the removal occurred after the critical period, it had no effect. This was the first demonstration of an endocrine function for nervous tissue in any animal. Unfortunately, this conclusion was not well accepted at the time because the brain was not believed to have the capacity to produce hormones. Not only did prevailing wisdom consider insects to be devoid of hormones, but the notion of the brain or any other nervous tissue as a source of hormones was unheard of. It was not even known at the time that neurons secrete chemicals at the synapse, so it is easy to understand how others were unwilling to accept that nerve cells could be secretory like an endocrine gland. The nervous and endocrine systems were viewed as functionally distinct in their roles of intercellular communication. It was not until the 1930s that Berta and Ernst **Scharrer** finally showed that the vertebrate brain had an endocrine function and also used insects as a convenient model system. Using the cockroach, they demonstrated that neurosecretory material moved from the cell bodies in the pars intercerebralis of the brain to

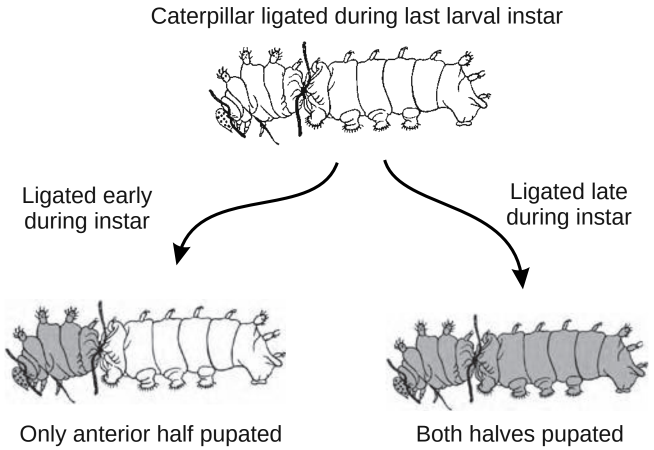


FIGURE 1.2. An experiment performed by Kopeć. When a caterpillar was ligated early during the last larval instar, only the anterior half later pupated. However, when ligated late during the last larval instar, both halves pupated. After Cymborowski (1992). Reprinted with permission.

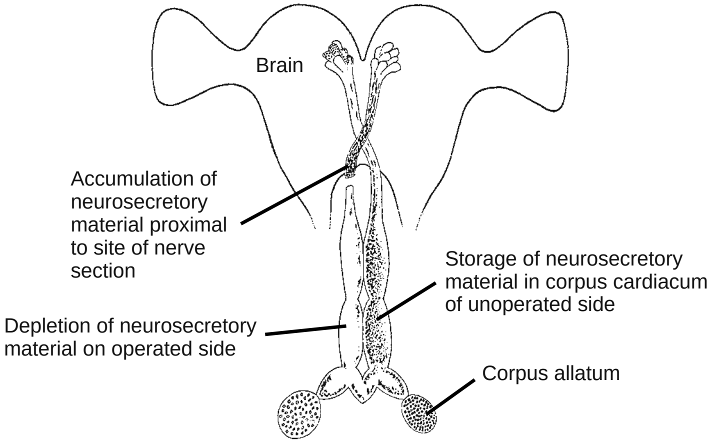


FIGURE 1.3. Evidence that neurosecretory material moves from the soma in the brain to the corpus cardiacum via axons. When the axon was severed, neurosecretory material accumulated anterior to the section. From Scharrer (1952). Reprinted with permission.

the corpus cardiacum, and that sectioning the neuron caused the material to accumulate proximal to the site of sectioning (Figure 1.3). The presence of neurosecretory cells in relatively primitive invertebrates suggested to them that the neurosecretory system was the initial means of intracellular communication from which the more specialized endocrine and nervous systems ultimately

evolved. Neurosecretory cells were not a late stage in evolution but rather an evolutionarily ancient means of biological communication.

At about this same time, **Wigglesworth** repeated the experiments of Kopeć using the blood-sucking bug, *Rhodnius prolixus*. *Rhodnius* has five larval instars, each of which requires a large meal of blood in order to molt. When fourth instar larvae were decapitated within 4 days after their blood meal, they failed to molt. However, when decapitation occurred later than 5 days following blood ingestion, the larvae did molt to the fifth instar (Figure 1.4). Because decapitation is far from precise and obviously removes a number of different structures located in the head, Wigglesworth next focused on the source of the endocrine effect by excising only a portion of the brain containing the neurosecretory cells. When

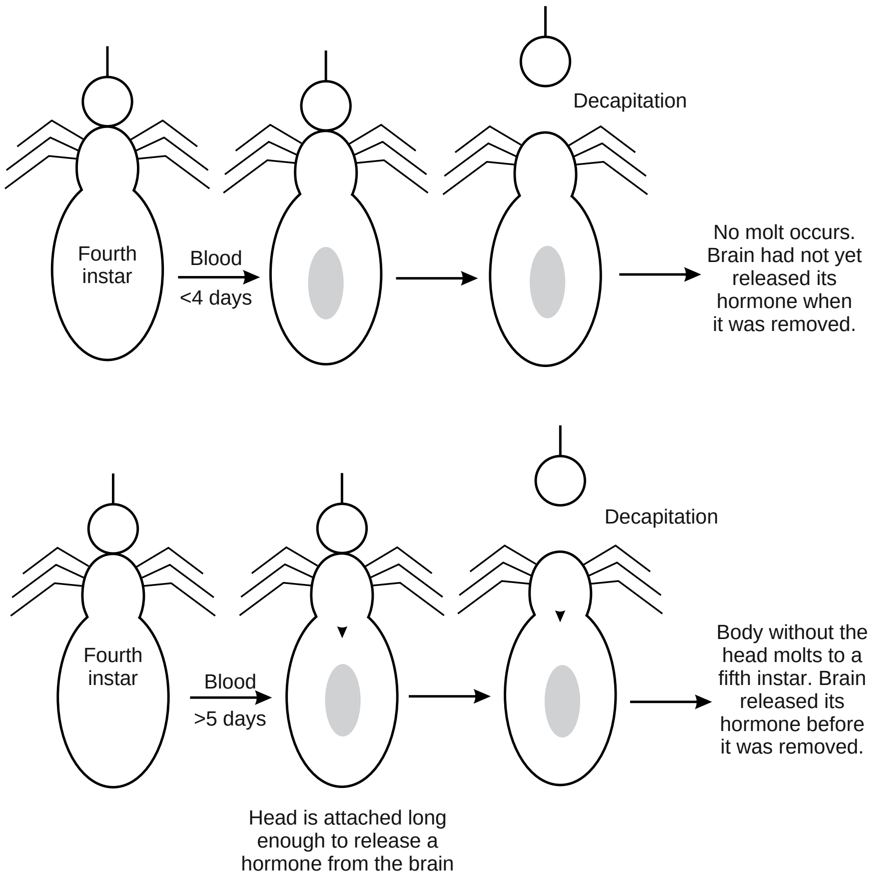


FIGURE 1.4. Wigglesworth's decapitation experiments using *Rhodnius* larvae. When fourth instar larvae were blood fed and decapitated within 4 days, they failed to molt. When they were decapitated after 5 days, the body still molted even though the head was not attached at the time.

these excised cells were implanted into the abdomens of other larvae that were decapitated early before the critical period, recipient larvae molted, demonstrating that neurosecretory cells were indeed the source of the brain's endocrine effect. The historical paths to additional insights into the existence of insect hormones will be discussed in the following sections that describe each hormone.

TYPE OF HORMONES IN INSECTS

Insects produce steroid hormones, such as **ecdysteroids**, sesquiterpenes that include all the **juvenile hormones**, and an abundance of **peptide hormones** produced by neurosecretory cells throughout the central nervous system and the midgut. There are also a number of **biogenic amines**, including octopamine, tyramine, and serotonin, that are primarily neurotransmitters derived from amino acids, but that also may have more widespread effects on the organism. Other hormones, such as **prostaglandin**, are derived from fatty acids. The circulating titers of a particular hormone and its ultimate effects on target cells are precisely modulated by the interplay between hormone synthesis, release, and degradation in the hemolymph once the hormone is released into circulation, and by the development and specificity of receptor sites on target tissues that allow the specific hormone to be recognized (Figure 1.5).

Peptide hormones are usually synthesized as larger precursor preprohormones and prohormones and then processed by proteolytic enzymes into the smaller final hormone (Figure 1.6). The peptide must be inserted into the cisterna of the endoplasmic reticulum, and a signal peptide portion must be attached in order for this to occur. The pre- and pro-portions are cleaved, and the peptide hormone is then released from the cell by exocytosis.

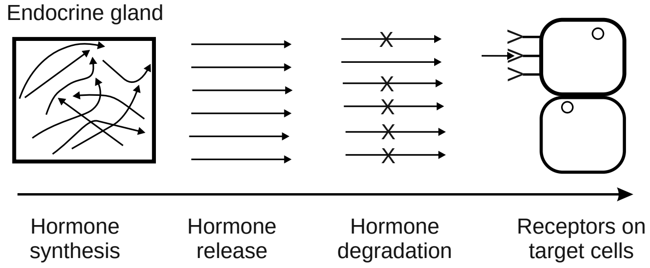


FIGURE 1.5. Factors that affect the activity of hormones. Hormonal activity in the circulatory system is regulated by its rate of synthesis by the endocrine glands, the rate of release into the blood, its degradation in the blood, and the development and presence of hormone receptors on target cells.

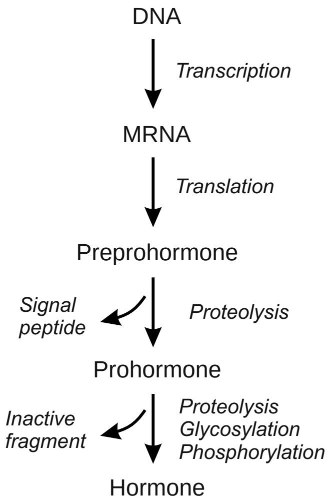


FIGURE 1.6. The synthesis and processing of peptide hormones.

Modes of Action

There are fundamental differences in the mechanisms by which different hormones act on target cells. Because of their nonpolar nature, ecdysteroids and juvenile hormones are able to enter the cell and bind to cytosolic and nuclear receptors, and ultimately they directly interact with DNA and its transcription (Figure 1.7). The nuclear receptors are ligand-dependent transcription factors that stimulate or block the synthesis of mRNA, and their presence makes the cell a target for the hormone. All the members of the nuclear receptor superfamily have a common modular structure (Figure 1.8A). It consists of an N-terminal A/B domain that is responsible for the ligand-independent transcriptional activation function that interacts with the transcriptional machinery of the cell and is responsible for isoform specificity, a highly conserved DNA-binding domain (DBD) that contains two zinc fingers, a hinge region (D), and a C-terminal ligand-binding domain (LBD). Some receptors may have an additional F domain at the extreme C-terminal end of unknown functional significance. The amino acid sequences of the receptor proteins can fold around zinc ions to form projections (Figure 1.8B), and because these zinc fingers provide the principal interface between the DBD and specific nucleotides within the hormone response element (Figure 1.8C), small differences in the amino acid residues of the receptor protein can affect the nature of the projections and the activity as a transcription factor. The various isoforms of each transcription factor may account for the tissue and target gene specificity of the hormones. The cell responds to

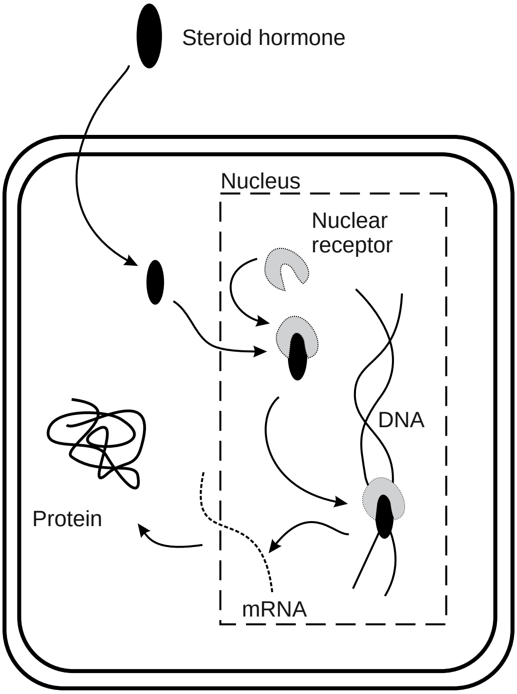


FIGURE 1.7. The mode of action of steroid hormones. The cell membranes are permeable to steroid hormones, so they pass through both the cell and the nuclear membranes. They bind to receptors that serve as transcription factors, so together they directly interact with DNA and regulate transcription of mRNA and the production of proteins.

the hormone by activating or inactivating specific genes when these transcription factors bind to hormone response elements of the target gene promoter.

In contrast, peptide hormones, which are much more polar, cannot pass through the cell membrane and must trigger a cellular response while remaining on the outside. The peptide hormones bind to protein receptors on the membrane's outer surface, altering the conformation of the receptor and consequently initiating the synthesis of **second messenger** molecules that carry the message inside the cell. These second messengers then act through a cascade of phosphorylations resulting in the activation or inactivation of specific enzymes. A small number of molecules of the first messenger, or hormone, can thus be amplified by the production of a larger number of these second messengers.

There are several different second messenger signal transduction systems, many of which involve a membrane-bound **G-protein** that consists of three subunits (α, β, γ) and operates between the first and second messengers. For some hormone transduction systems, the second messenger is **cyclic AMP**

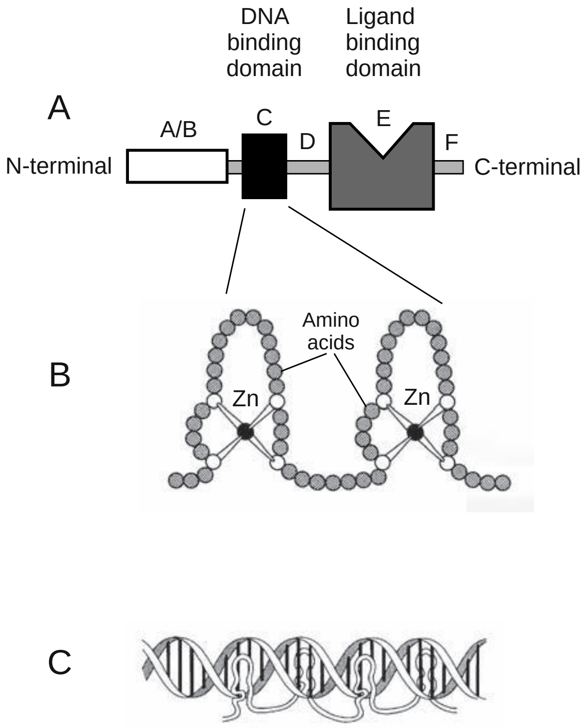


FIGURE 1.8. Characteristics of nuclear receptors. A. Modular structure of domains. B. Amino acids form fingers that fold around zinc ions. C. Binding of zinc finger transcription factors to hormone response elements.

(Figure 1.9). When the membrane receptor for the hormone becomes bound, it changes its conformation and causes it to come in contact with the G-protein. This causes the G-protein subunits to dissociate, with the α subunit activating the membrane-bound enzyme **adenylate cyclase** and forming cyclic AMP (cAMP) from ATP. The cAMP that is formed then stimulates a protein kinase that phosphorylates and activates enzymes and ribosomal and nuclear proteins to elicit a biological response (Figure 1.10A).

A second major signal transduction pathway coupled to G-proteins involves the activation of a phospholipase and a subsequent increase in intracellular calcium. The hormone-receptor complex acts through a G-protein to activate a membrane-bound phospholipase that hydrolyses the complex membrane molecule, phosphatidylinositol 4,5-diphosphate (PIP_2) to form two second messengers, triphosphoinositol (IP_3) and diacylglycerol (DAG). The IP_3 causes the release of calcium from the endoplasmic reticulum that can activate exocytosis in cell secretory mechanisms and cell enzyme cascades. The DAG activates a

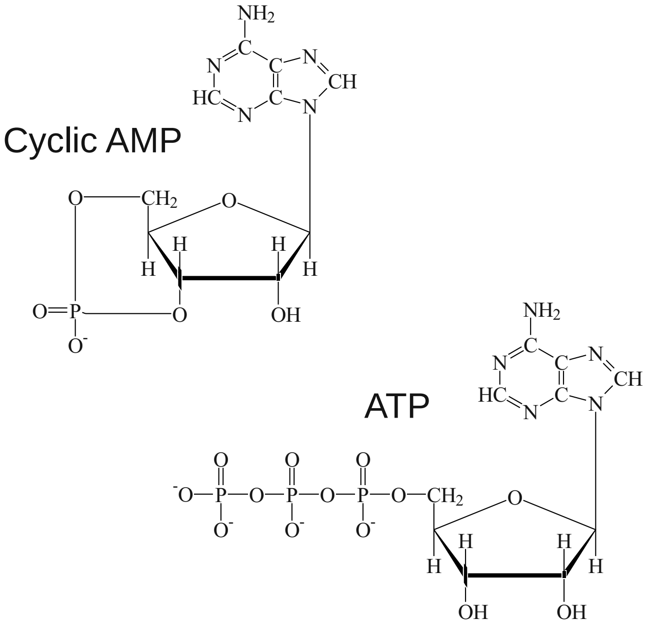


FIGURE 1.9. Two of the major roles of adenine in cells. As cyclic AMP, it acts as a second messenger in cells. As ATP, it serves as a form of energy storage and transfer.

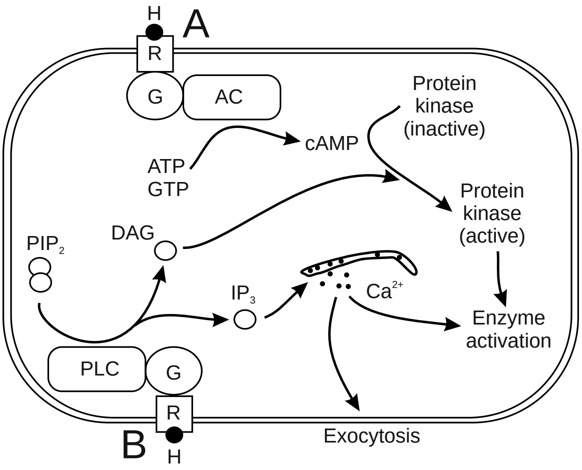


FIGURE 1.10. Signal transduction via second messengers. A. A protein kinase is activated by the second messenger cAMP that is formed from the adenylate cyclase (AC) generated when the G-protein dissociates as the hormone binds to the membrane-bound receptor. B. The two second messengers, triphosphoinositol (IP3) or diacylglycerol (DAG), release calcium or activate a protein kinase, respectively, that can then activate enzymes. The second messengers are formed when a phospholipase (PLC) is activated from the binding of the hormone (H) to the receptor-associated G-protein.

membrane-bound protein kinase that phosphorylates and activates other enzymes (Figure 1.10B). In both these pathways, preexisting enzymes are activated when the hormone binds, unlike the mechanism of steroid hormone action that directly involves the activation of gene transcription and the synthesis of new enzymes. G-proteins may also influence the opening of membrane channels that allow Ca^{2+} or K^{+} to enter the cell.

The gas, **nitric oxide** (NO), can also serve as a second messenger in insect systems. Increases in intracellular calcium from intracellular stores or through Ca^{2+} membrane channels activate the enzyme **nitric oxide synthase** through the calcium binding protein **calmodulin,** forming nitric oxide and citrulline from arginine (Figure 1.11). The nitric oxide is able to cross the cell membrane and activate a soluble guanylate cyclase in neighboring cells that increases levels of cyclic GMP (cGMP). The cGMP has a wide variety of effects on the target cell, including the activation of cGMP-dependent enzymes and the permeability of

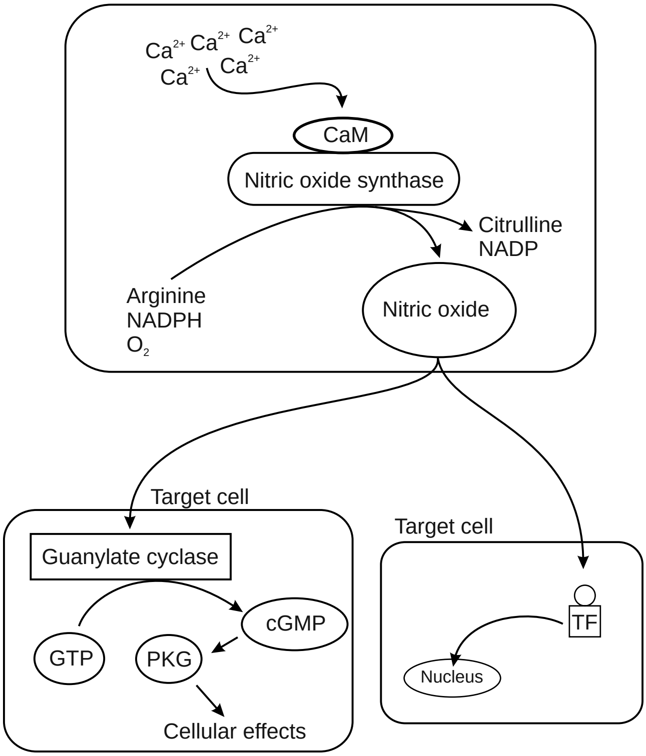


FIGURE 1.11. Nitric oxide as a second messenger. The enzyme nitric oxide synthase can be activated by several pathways. The nitric oxide formed in the cells diffuses easily through the cell membrane and is able to activate the enzyme guanylate cyclase, causing a rise in cGMP that then has cellular effects. It can also bind to transcription factors (TF) and affect gene expression (CaM, calmodulin).

membrane channels. NO can also bind to nuclear transcription factors and repress or induce gene transcription. Different isoforms of nitric acid synthase may exist in a single cell, each responsible for a different target. NO signaling in insects is associated with the Malpighian tubules, salivary glands, the central nervous system, and the development of the compound eyes. As in vertebrates, NO signaling is important in chemosensory transduction, especially in olfactory receptors associated with the antennal lobes of insects. NO signaling is also involved in establishing patterns of neural outgrowth and migration and influence neural wiring.

PROTHORACICOTROPIC HORMONE

Prothoracicotropic hormone (PTTH) was the first insect hormone to be discovered but the last major hormone to be structurally identified. This was the brain hormone of Kopeć's early investigations, but because the brain was later found to produce so many hormones, the simple designation of "brain hormone" was no longer descriptive. Its current name underscores its ability to activate the prothoracic glands. Wigglesworth's work in the 1940s established that a region of the *Rhodnius* brain containing large neurosecretory cells was the source of PTTH activity. Two pairs of neurosecretory cells were specifically identified in *Manduca* by the microdissection and implantation experiments of **Agui** and coworkers in 1979. **Williams** demonstrated the relationship between the brain and prothoracic glands in the late 1940s and early 1950s. He implanted both the prothoracic glands and a brain from a chilled pupa into a diapausing pupa and showed that both the brain and prothoracic gland were required to terminate diapause and that the brain activated the prothoracic glands. When he implanted a single chilled brain into a chain of brainless diapausing pupae connected by parabiosis, all the pupae successively underwent adult development (Figure 1.12). PTTH is produced in the lateral neurosecretory cells of the brain and is released in the corpus cardiacum that terminates in the wall of the aorta or, in some insects, is released by the corpus allatum. PTTH acts on the prothoracic gland to regulate the synthesis of ecdysteroids.

The delay in its structural identification was largely the result of the lack of a reliable bioassay. Early bioassays for PTTH consisted of debrained pupae, referred to as "dauer" (German: *a long time*) pupae because they could survive for 2 to 3 years until all the nutrients within them had been exhausted. When extracts with PTTH activity were injected, the pupae initiated metamorphosis to the adult stage. There were several problems with this bioassay, including a low reproducibility due to physiological variations between pupae and the relatively long time it took to score a response. A more direct assay for PTTH was developed by **Bollenbacher** and coworkers in 1979 using the criterion of ecdysone production by a pair of prothoracic glands that were maintained *in vitro*. The basal rate of ecdysone synthesis by a nonstimulated gland is compared to

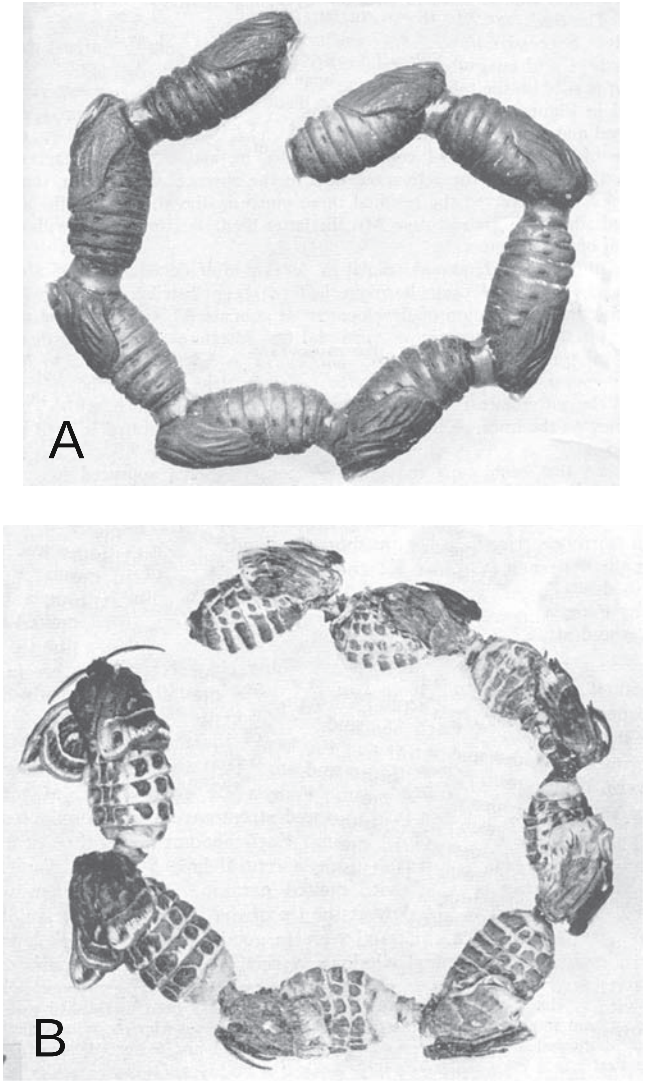


FIGURE 1.12. An experiment by Williams where a chain of brainless parabiosed pupae (A) were activated to molt by the implantation of a single brain into the first pupa (B). From Williams (1952). Reprinted with permission.

the rate of ecdysone secretion by a gland that is incubated with a suspected source of PTTH. The activation of the gland is indicated by a significant increase in ecdysone synthesis, measured by a radioimmunoassay (Figure 1.13). It is still not a completely satisfactory bioassay because the prothoracic gland preparations that are required involve a sometimes difficult dissection and isolation.

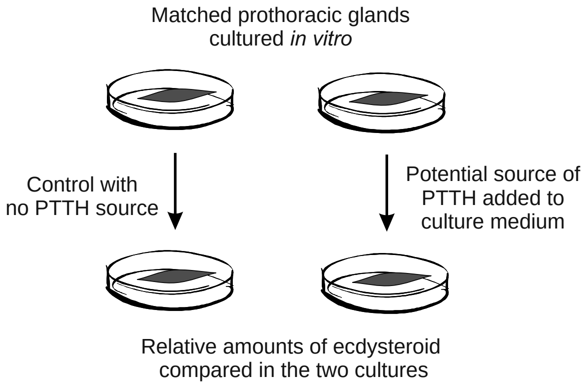


FIGURE 1.13. An assay for PTTH developed by Bollenbacher et al. (1979). A pair of matched prothoracic glands are removed from the insect and placed in culture. If PTTH is added to the culture, the glands produce increased amounts of ecdysteroids into the medium.

The PTTHs from only a handful of insects have been identified, with most of the work centered on the lepidopterans *Bombyx mori* and *Manduca sexta*, and the dipteran *Drosophila melanogaster*. Initial isolations characterized the PTTHs as multiple forms that fell into two groups: the "big" PTTH (14–29 kDa) and the "small" PTTH (3–7 kDa) were based on a rather serendipitous bioassay. *Bombyx mori* silkworm moths were plentiful because of rearing methods developed by the silk industry and were ideal sources of the hormone because so much biomass was required as a starting point for the isolation of the minute quantities of hormones. In contrast, surgery for brain removal is easier in the *Samia cynthia* moths, and they made ideal bioassay animals for testing the PTTH activity of introduced materials. The standard bioassay thus involved attempts to identify PTTH from *Bombyx* bioassayed in a debrained *Samia*, which then initiated metamorphosis if PTTH was present. The small PTTH isolated from *Bombyx* was indeed able to activate the prothoracic glands of the related moth, *Samia cynthia*, as well as those of the blood-sucking bug, *Rhodnius prolixus*, but curiously, was unable to activate the glands of *Bombyx*. This small PTTH was renamed **bombyxin** and is no longer considered as a true PTTH, mainly because there is no relationship between its titer in the hemolymph of *Bombyx* and the levels of 20-hydroxyecdysone that result, making it a non-PTTH. The multiple molecular species of the bombyxins that have been identified appear to share some homology with vertebrate insulin, but their exact roles in insect systems have yet to be determined. Bombyxin receptors are present on the ovaries of some lepidopterans, and the hormone may be involved in ovarian development and the utilization of carbohydrate during egg maturation. Insulin-like molecules have been implicated in the control of insect growth. The big PTTH has the true PTTH activity: it stimulates the prothoracic glands to

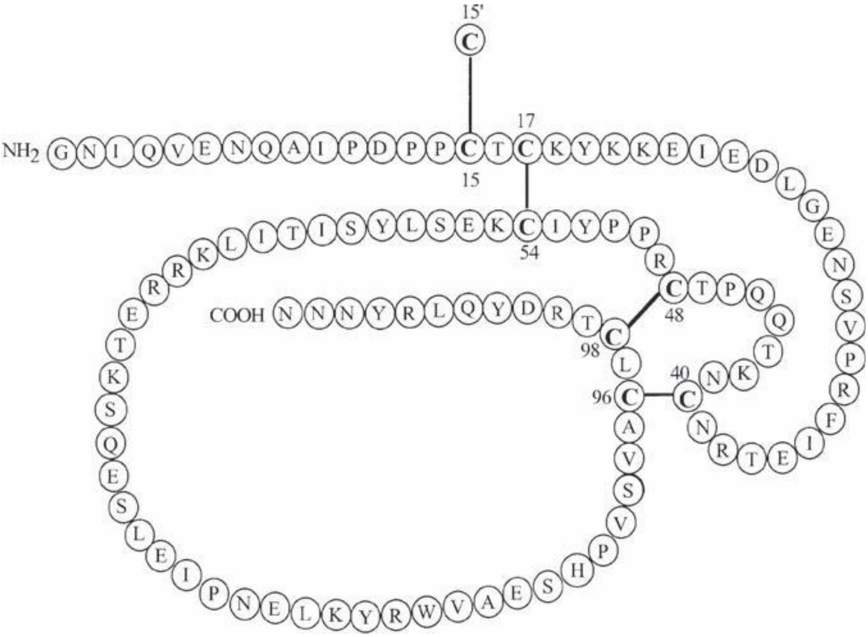


FIGURE 1.14. The amino acid structure of PTTH. Only one of the two identical chains in the homodimer is shown. From Nagata et al. (2005). Reprinted with permission.

produce ecdysone. In *Bombyx*, big PTTH is synthesized as a large 224 amino acid precursor and then cleaved to liberate a 109 amino acid subunit. The active molecule is a homodimer, consisting of two identical chains that are held together by disulfide bonds (Figure 1.14). The folding of the molecule is largely controlled by its intra- and intermolecular disulfide bonds.

Control of PTTH Release and Its Mode of Action

The **corpus cardiacum** (CC) was originally considered to be the neurohemal organ for PTTH release in all insects until it was later shown that some insects used the corpus allatum. In any event, the CC is the major neurohemal organ in insects and releases a large number of neuropeptides. It may be a paired or single structure and lies posterior to the brain, closely associated with the aorta (Figure 1.15). It contains the axon terminals from both the lateral (LNC) and the medial (MNC) neurosecretory cells of the brain, which are refered to as extrinsic because the neurosecretory cell bodies lie elsewhere. There are also intrinsic neurosecretory cells that have both their cell bodies and axons located entirely within the corpus cardiacum. There are often two lobes that compose

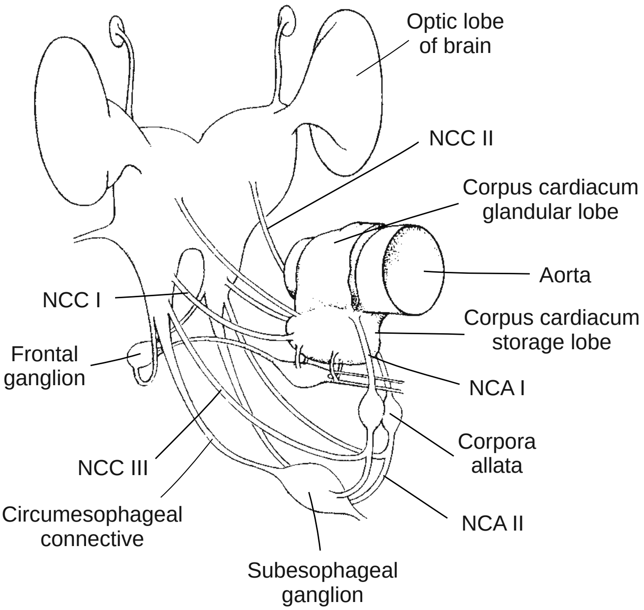


FIGURE 1.15. The lobes of the corpus cardiacum of the locust and their innervations. From Veelaert et al. (1998). Reprinted with permission.

the CC, consisting of the storage lobe derived from extrinsic cells and the glandular lobe made up of intrinsic cells. It is innervated by the corporis cardiacum I from the MNC, the corporis cardiacum II from the LNC, and the corporis cardiacum III from the tritocerebrum. In larval dipterans, it is incorporated into the ring gland along with the prothoracic gland and the corpus allatum. In addition to PTTH, the CC releases an ovarian ecdysteroidogenic hormone in mosquitoes, adipokinetic hormone, several neuroparsins and myotropins, and the pheromone biosynthesis activating neuropeptide in other insects.

It is the release of PTTH that determines the occurrence of the molt by activating the prothoracic glands to produce the ecdysteroid molting hormone. Most insects release PTTH from their neurohemal organ based on the receipt of environmental stimuli, which may include photoperiod, temperature, and nervous stimuli. For example, in *Rhodnius*, where molting follows blood ingestion, the abdominal distention resulting from a large blood meal triggers stretch receptors that then send a message to the brain to release PTTH. Neither a small meal nor a series of small meals is able to trigger molting; the blood volume must exceed a critical threshold to activate the stretch receptors that, through the central nervous system, initiate PTTH release. The nutritive capacity of the

blood is not important, because large meals of saline can also provoke a molt. In the lepidopteran, *Manduca sexta*, PTTH release is regulated by photoperiod, occurring during a circadian window. The failure of PTTH to be released is the cause of the pupal diapause in some lepidopterans. Without PTTH and the resulting ecdysteroid, the pupa cannot develop further and molt to the adult stage. In *Hyalophora cecropia* moths, pupal diapause can be terminated by a prolonged exposure to cold temperatures followed by a warming.

Because PTTH is a peptide hormone, it is unable to enter the cells of the prothoracic gland and must exert its influence from the outside through a G-protein coupled receptor. This G-protein coupled receptor has yet to be identified, but its activation increases intracellular Ca^{2+} as a second messenger, which then activates protein kinases that can phosphorylate and activate enzymes in the biosynthetic pathways that lead to a cellular response (Figure 1.10B).

ECDYSTEROIDS

Ecdysteroids are the generic name for a group of related steroid hormones that are primarily involved in the molting process of arthropods but also have wide-ranging effects in every developmental stage. The experiments by **Kopeć** and **Wigglesworth** demonstrated the importance of the brain in the molting process, but it was **Hachlow** in 1931 who first showed that the brain does not act alone. When lepidopteran pupae were cut at different points along the body and the cut ends sealed, only the parts that contained the thorax developed adult characteristics. This suggested that an organ in the thorax was also necessary for molting and metamorphosis. **Fukuda**, in 1940, demonstrated that the ecdysone-secreting organ was the prothoracic gland. In double ligation experiments using last instar silkworm moth larvae, Fukuda observed that only those portions ligated anterior to the prothoracic gland pupated, and when the prothoracic gland was implanted into the posterior portions, those also underwent pupation. Along with the experiments by **Williams** mentioned in the previous section, these results established that both the brain and prothoracic glands released factors necessary for a molt to occur, with the brain activating the prothoracic glands to produce the molting hormone. The x-ray crystallography of **Huber** and **Hoppe** in 1965 identified the structure of the ecdysone molecule, and the definitive demonstration that the prothoracic glands produce ecdysteroids was accomplished independently by **King** and coworkers and **Chino** and coworkers. **Hagedorn** and coworkers established the role of this hormone in insect reproduction in 1975 when they demonstrated that the ovaries of mosquitoes produced ecdysteroids during egg maturation.

Ecdysone was the first insect hormone to be structurally identified, an accomplishment that became possible only once an assay for its biological activity was developed. The *Calliphora* bioassay that **Fraenkel** devised in 1935 consisted of

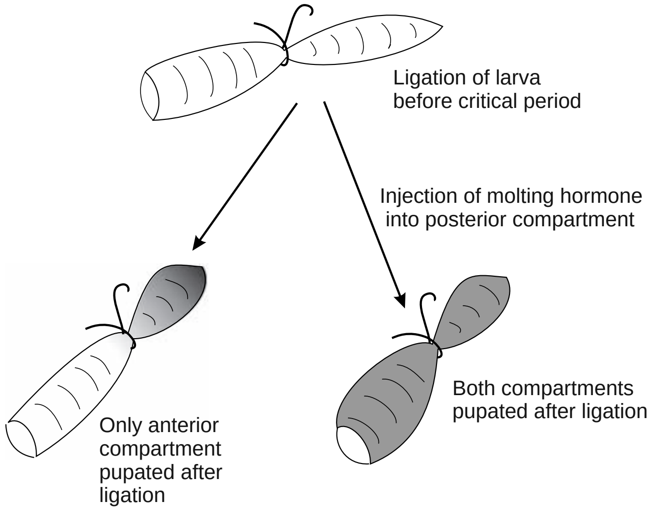


FIGURE 1.16. The *Calliphora* bioassay developed by Fraenkel (1934). When substances with molting hormone activity were injected into the posterior compartment of ligated *Calliphora* pupae, the cuticle of the posterior compartment underwent a molt along with the anterior compartment.

fly larvae that were ligated during their last larval instar. Because their posterior portions lacked any molting hormone, they failed to pupariate, unlike the anterior portion that contained the necessary endocrine centers. Pupariation in the posterior portion was induced when extracts containing the molting hormone or suspected molting hormone activity were injected (Figure 1.16). With a sensitivity of between 5 and 50 ng per abdomen, this bioassay was the basis for measuring the success of the first isolation of ecdysone. Radioimmunoassay is currently the most common technique for the detection and analysis of ecdysteroids. Ecdysteroids by themselves are not immunogenic, and to obtain antibodies derived from immunization, the hormones must first be coupled to a protein. Although sensitive, it lacks specificity in determining which of the biologically active ecdysteroids may be present. Several other ecdysteroid bioassays have been used, including the *in vitro* determination of chromosome puffs and the morphogenesis of imaginal discs.

Butenandt and **Karlson** purified 25 mg of the hormone starting with approximately 500 kg (a half ton!) of *Bombyx mori* pupae. Shortly afterward, a second, more polar substance with molting hormone activity was isolated, and the two hormones were named **α- and β-ecdysone**, respectively. Other ecdysteroids have since been isolated, and the convention was established to use "ecdysteroid" as the generic name for the group. The first ecdysteroid to be identified, α-ecdysone, is now referred to as **ecdysone**. The second hormone, β-ecdysone, is now referred to as **20-hydroxyecdysone** (20HE) and is hydroxylated from

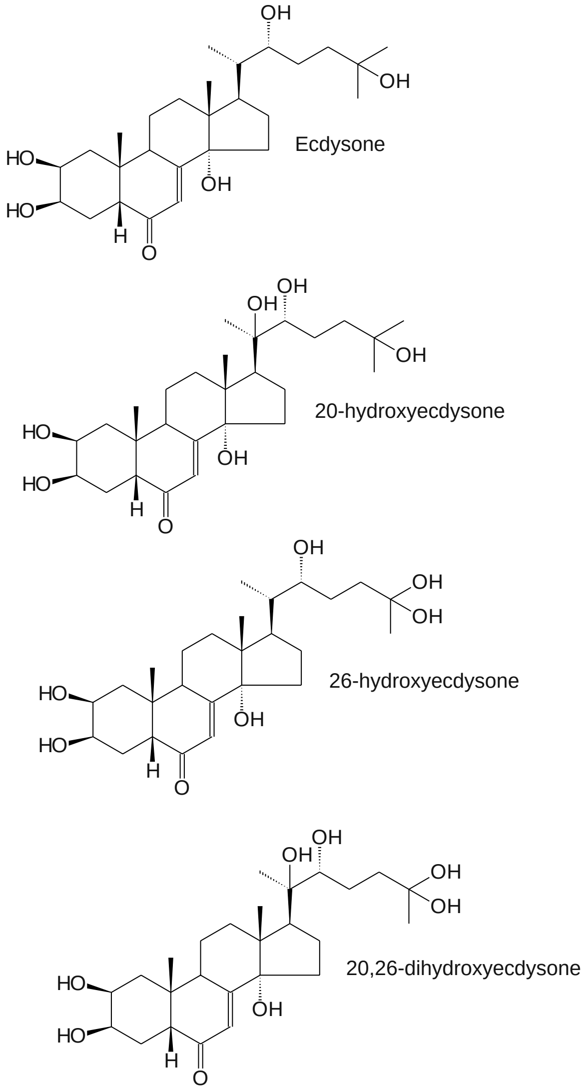


FIGURE 1.17. Some common ecdysteroids.

ecdysone by target tissues (Figure 1.17). It is the true molting hormone in that it is most active in inducing a molt. There are more than 300 different analogs of the molting hormone with more than 70 of these found in insects. The diversity of this group of steroid hormones is the result of the many variants in the number and positions of hydroxyl groups on the skeleton that also may be

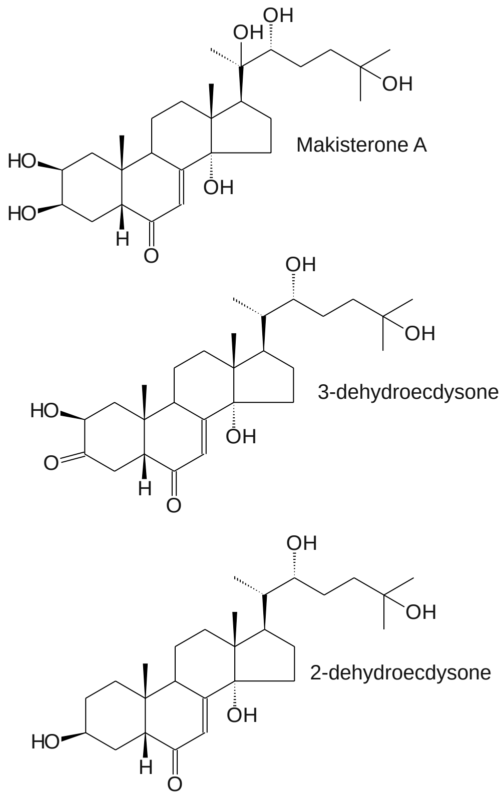


FIGURE 1.17. *Continued.*

conjugated to other groups. Many of these are **phytoecdysteroids**, produced in plants at much higher concentrations than the ecdysteroids of insects, sometimes as high as 3% dry weight. Although they may simply represent metabolic intermediates, these phytoecdysteroids may be feeding deterrents or toxic substances that affect the survival of insect herbivores. Most of the ecdysteroids that are commercially available for experimentation are phytoecdysteroids.

Identification, Synthesis, and Control of Ecdysteroid Production

Ecdysteroids were expected to be similar to vertebrate steroid hormones in their solubility, but this expectation delayed their successful isolation. Given the many

hydroxyl groups on the ecdysteroid molecule, one face is relatively hydrophilic and thus poorly soluble in the organic solvents that were used to extract the generally lipophilic vertebrate steroids. Ecdysone is a steroid hormone belonging to the class of substances known as terpenoids, along with the juvenile hormones. All terpenoids are synthesized by the combination of at least two isoprene precursors that are responsible for the production of many important biological agents in plants and animals. Similar to the synthesis of juvenile hormones, isoprene must be first activated to isopentenyl pyrophosphate and dimethylallyl pyrophosphate via the mevalonate pathway. Lacking the enzyme squalene synthase, arthropods do not maintain the pathway from farnesyl pyrophosphate that generates cholesterol (Figure 1.18).

The precursors for ecdysteroid synthesis by the prothoracic gland of insects are sterols, such as cholesterol (Figure 1.19). Although most organisms can synthesize cholesterol from acetate precursors through the series of isoprene building blocks, arthropods cannot do this and require cholesterol in their diets. Zoophagous insects can easily ingest sufficient cholesterol, which is a major animal steroid. Phytophagous insects instead encounter campesterol, sitosterol, or stigmasterol (Figure 1.19), the major sterols in plants that contain additional methyl and ethyl groups on their side chains, and the insects must dealkylate them to

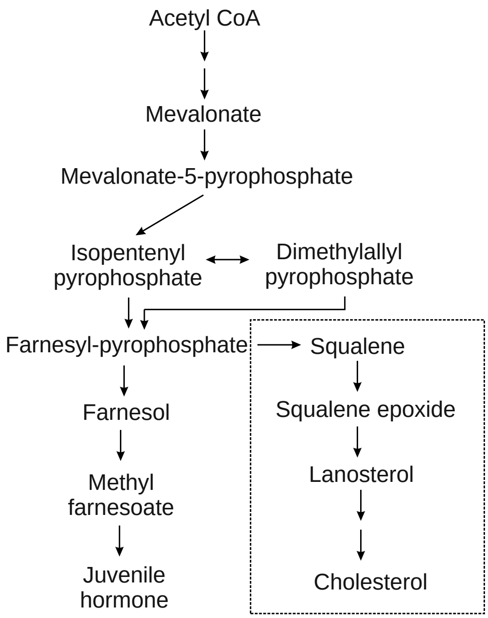


FIGURE 1.18. The isoprene pathway toward the synthesis of JH. Components within the box are not present in insects.

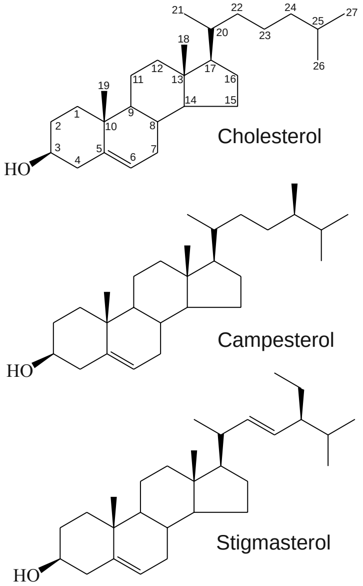


FIGURE 1.19. Cholesterol and the system of numbering its carbon atoms. Two major plant sterols ingested by phytophagous insects.

form cholesterol. Those phytophagous insects unable to make the conversion — including many hemipterans, the honey bee, and some dipterans — produce makisterone A, or 24-methyl 20-hydroxyecdysone, as their molting hormone (Figure 1.17). This is the only 28 carbon ecdysteroid; all the rest are 27 carbon sterols. Although the major steps in the ecdysteroid biosynthetic pathway in insects are known, the complete identification of all intermediates has yet to be described. Their detection has been difficult because the intermediates do not accumulate to any degree during ecdysteroid biosynthesis.

Important advances in the understanding of the steps of ecdysone synthesis were made with the identification of a group of genes in *Drosophila* placed into the Halloween family. The Halloween genes are associated with a failure to develop beyond the embryonic stages when mutated, and the resulting defects appear to result from deficiencies in ecdysone production. The steps of ecdysteroid biosynthesis include a series of hydroxylations involving several of these Halloween genes, whose gene products have been identified as P450 enzymes that are bound to the mitochondria and endoplasmic reticulum of the prothoracic glands. These genes include *phantom* (*phm*) that codes for the 25-

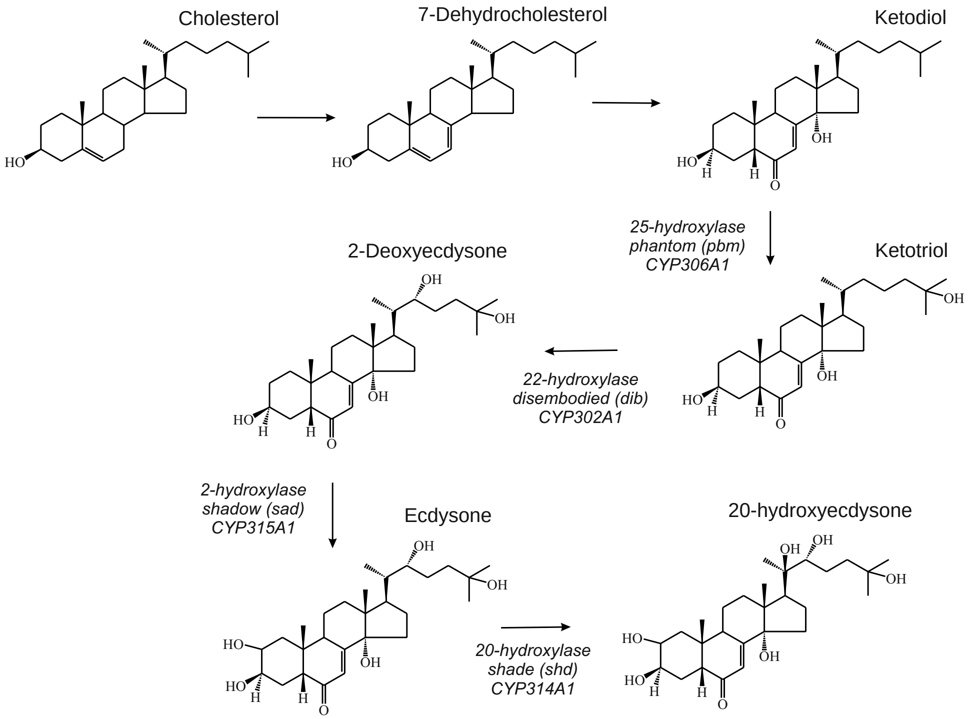


FIGURE 1.20. The steps, genes, and enzymes involved in the synthesis of 20-hydroxyecdysone from ingested cholesterol.

hydroxylase enzyme CYP306A1; *disembodied* (*dib*), whose gene product is the 22-hydroxylase enzyme CYP302A1; and *shadow* (*sad*) that encodes the 2-hydroxylase enzyme CPY315A1. The s*hade* (*shd*) gene produces the enzyme 20-monooxygenase, a 20-hydroxylase that converts ecdysone to 20HE. These are key enzymes associated with the prothoracic gland that catalyze the final steps in ecdysteroid biosynthesis (Figure 1.20). Enzymes that catalyze other steps in ecdysone synthesis have yet to be identified.

The primary site of ecdysteroid synthesis is the prothoracic gland, which develops during embryogenesis from ectodermal cells in the head, and in some insects remains there to be known as ventral glands. In cyclorraphan Diptera, the prothoracic gland has been incorporated into a ring gland that also consists of the corpus allatum and corpus cardiacum (Figure 1.21). In other insects, the glands are found in the thorax where they form loose chains of cells, with a close association with the trachea, which has led to them often being called peritracheal glands (Figure 1.22). Nervous innervations of the gland consist of a pair of nerves from the subesophageal ganglion and sometimes

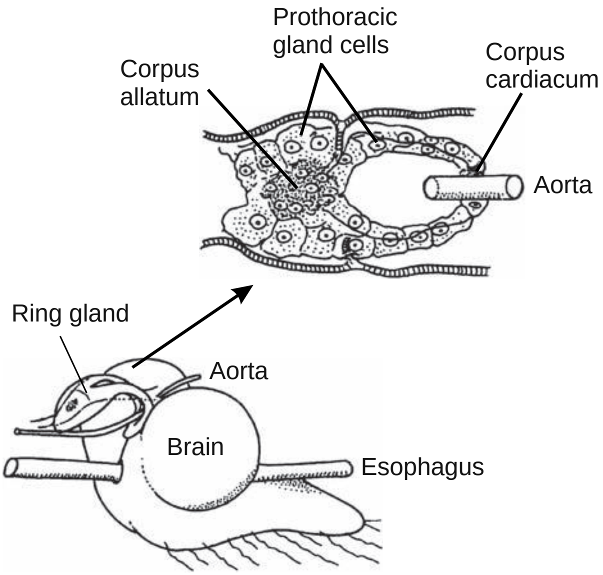


FIGURE 1.21. The ring gland of higher dipterans, consisting of the corpus cardiacum, corpus allatum, and the prothoracic gland assembled in a ring structure. From Wigglesworth (1984). Reprinted with permission.

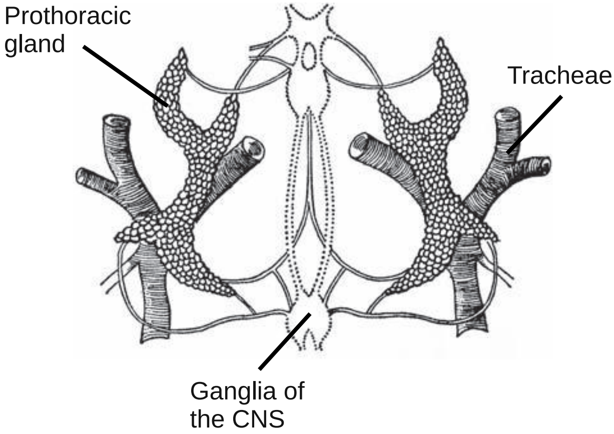


FIGURE 1.22. Location of the prothoracic glands around the thoracic tracheae. From Cymborowski (1992). Reprinted with permission.

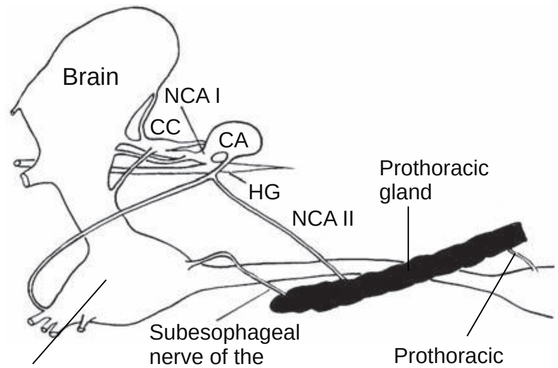


FIGURE 1.23. Innervation of the prothoracic gland. From Beaulaton (1990). Reprinted with permission.

additional nerves that issue from the prothoracic and mesothoracic ganglia (Figure 1.23). In spite of the nervous connections, the primary mode of gland activation is hormonal.

Because adult pterygote insects no longer molt, the prothoracic gland is not necessary during this developmental stage. It degenerates in most adult pterygote insects by **apoptosis**, a programmed cell death, as a consequence of the hormonal conditions present during the last molt. The stimulus for prothoracic gland degeneration is its exposure to ecdysteroids in the absence of juvenile hormone, the conditions present during metamorphosis. This explanation is not completely satisfactory, however, because the prothoracic glands from *Manduca* fail to undergo apoptosis in a JH-free culture that contains ecdysteroids. The gland persists for several days in adult *Periplaneta americana* and does not degenerate at all in gregarious female *Schistocerca* adults, although these glands can no longer produce ecdysteroids when they are cultured *in vitro*. Apterygote insects, which continue to molt as adults, retain their active prothoracic glands.

The active form that binds to cellular receptors is 20-hydroxyecdysone, converted from ecdysone by target tissues. This raises some questions about which of these is truly considered to be the molting hormone: ecdysone released by the endocrine gland or 20HE acting on target tissues. In some lepidopterans, the ecdysteroid that is released from the prothoracic gland is 2-dehydroecdysone that is then converted to ecdysone in the hemolymph. There is no evidence that the hormone is stored in the prothoracic gland, and it appears to be released when it is synthesized. Once released, the hormone circulates both alone and as bound to carrier proteins. The bound form is inactive and may serve as a reservoir of the hormone.

Metabolism and Degradation of Ecdysteroids

There are several routes of ecdysteroid inactivation that may vary depending on the species, tissue, and developmental stage. Ecdysteroids may be converted into phosphate or fatty acyl ester conjugates, converted into the ecdysonoic acid, or transformed into 3-epi (3α)-ecdysteroid (Figure 1.24). The predominant mode of inactivation appears to be phosphoconjugation. The first ecdysteroid conjugate to be identified was the ecdysone 3-acetate from grasshopper embryos. Maternal ecdysteroids may also be inactivated as phosphate glucoside and sulfate conjugates

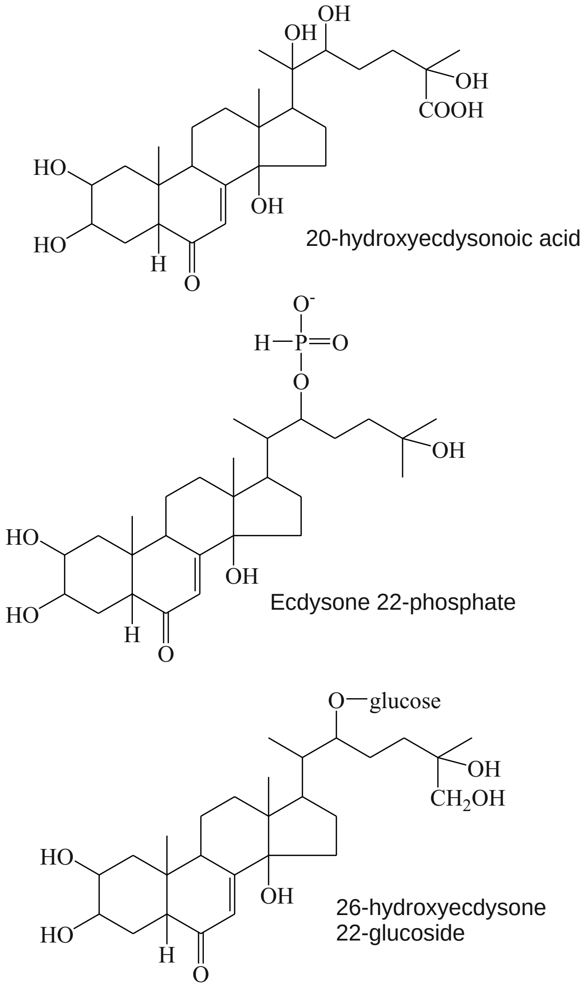


FIGURE 1.24. Some of the products of ecdysone inactivation.

and is passed into the eggs for future use as maternal storage forms of the ovarian hormone. Fatty acid ecdysteroid conjugates may be bound to yolk proteins, and the degradation and utilization of these yolk proteins during embryogenesis releases the conjugated ecdysteroids. When the ecdysteroids are released from the conjugates during embryogenesis, they become available for activity. This is most apparent in the embryos of the silkworm *Bombyx mori*, where 20HE is necessary for embryonic development and a 20HE deficiency leads to an embryonic diapause in which development ceases. Nondiapausing eggs are able to release the ecdysteroid-phosphate conjugates from yolk granules, and free ecdysteroids are subsequently released from the conjugates.

Control of Ecdysone Secretion by the Prothoracic Glands

The classical model of ecdysone secretion involves the prothoracic glands becoming activated in response to circulating PTTH, but the system appears to be much more complex than that. PTTH does indeed activate cAMP as a second messenger in prothoracic gland cells but several **FMRFamide-related peptides** (FaRP) additionally modulate this secretion by way of the nervous system. The FaRPs prevent ecdysone secretion by reducing cAMP, arriving at the prothoracic glands not through the hemolymph as a conventional hormone but instead via release by neurons that directly innervate the glands. The insect-like peptide bombyxin, which affects the nutrient-dependent growth of insect tissues, causes the prothoracic glands to grow, and because larger glands are more likely to produce ecdysone at a given body weight than are smaller glands, bombyxin ultimately determines the point at which the prothoracic glands will respond. In addition, two inhibitory peptides modify the secretion of ecdysone that is stimulated by PTTH. A **myoinhibitory peptide/prothoracicostatic peptide** is released from hindgut neurosecretory cells stimulated by the ecdysone peak. A **myosuppressin** released by the brain may suppress ecdysone production during the intermolt (Figure 1.25).

Other Sources of Ecdysteroids

The prothoracic gland is not the only source of ecdysteroids. Even though the prothoracic gland degenerates in adult insects, ecdysteroids still occur in their hemolymph. In these adults, the site of ecdysteroid synthesis has been shifted to the ovaries and the testes. In many female insects, ecdysteroids are produced by the follicle cells of the ovaries, where they are conjugated to other molecules and incorporated into the eggs for later use during embryogenesis. Developing insect embryos contain several different ecdysteroids in both free and conjugated

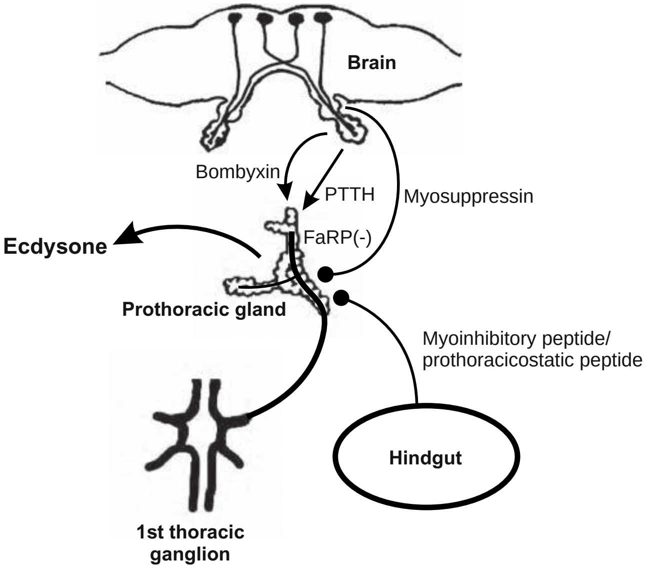


FIGURE 1.25. Model for the control of prothoracic gland secretion. From Truman (2006). Reprinted with permission.

forms, including 20, 26-dihydroxyecdysone and 26-hydroxyecdysone (Figure 1.17) that appear in the embryo well before its synthetic machinery could be responsible. However, the existence of lethal embryonic Halloween mutations suggest that some ecdysteroid biosynthesis also may occur during embryogenesis. Ovarian ecdysteroids may be released into the hemolymph and act on the fat body to activate the synthesis of yolk proteins. Ecdysteroids can be produced in males of several species by the larval and pupal sheaths of the testes. The epidermal cells may also be a source of ecdysteroids during certain developmental stages. A discussion of these roles of ecdysteroids will be found in the chapters on reproductive and developmental systems.

Synthesis of ecdysteroids by the prothoracic gland of an insect larva occurs by the action of PTTH. Its main sites of production are the neurosecretory cells of the brain but PTTH activity has also been identified in the subesophageal ganglion and the ganglia of the ventral nerve cord. Synthesis of ecdysteroids during other developmental stages by additional sources such as ovaries and testes occurs in response to other ecdysiotropic neurohormones. **Ovarian ecdysiotropic hormones**, a **testes ecdysiotropin**, and **ecdysiostatins** produced by neurosecretory cells of the brain modulate ecdysteroid production by these organs. Insulin has been implicated in insect growth, and it is intriguing that *Manduca* prothoracic glands also express an insulin receptor. Examinations of neural control of the prothoracic gland have yielded conflicting data, showing both inhibition and stimulation by nerves.

Mode of Action of Ecdysteroids

Ecdysteroids, as typical steroid hormones, can easily diffuse into cells. They directly affect gene expression, causing the activation or inactivation of certain genes and the resulting synthesis or inhibition of enzymes and other regulatory peptides. The evidence that ecdysteroids influence gene transcription comes from studies of polytene chromosomes in *Drosophila*, which are chromosomes that have replicated but whose strands have not separated. Their alignment of replicated DNA makes a banding pattern visible with light microscopy. The **puffs** that are sometimes visible represent sites of active gene transcription, and puffing patterns are correlated with the developmental stage of the insect as well as the ecdysteroid titers. There is a characteristic sequence of puffs in the polytene salivary gland chromosomes of last larval instar *Drosophila* that is induced by an ecdysteroid pulse late in the instar. A few early puffs are rapidly induced by the hormone and then regress, followed by a large number of late puffs that persist through the formation of the puparium. Inhibitors of protein synthesis do not affect the formation of early puffs, suggesting they are responding directly to the hormone, but these inhibitors do prevent their later regression. Inhibitors of protein synthesis also prevent the induction of late puffs, but these can be induced even when hormone is withdrawn once the early puffs are formed. The puffing pattern that is normally characteristic of late third instar *Drosophila* larvae can be prematurely induced by injecting early third instar larvae with ecdysteroids. The injected hormone is localized in the cell nucleus and can be identified binding to the inducible puff site.

Based on these and other observations, a model for the action of ecdysteroids on gene transcription was developed by **Ashburner** in 1974 and has since been enhanced with the identification of specific genes that are known to be activated during the puffing sequence (Figure 1.26). In the model, ecdysteroids bind to an ecdysteroid receptor that consists of a heterodimer assembled by the products of the genes *EcR* and *USP* and that acts as a DNA binding protein and nuclear receptor. Gene expression is activated by the binding to specific hormone response elements in the promoter region of target genes. The *EcR* gene is induced directly by ecdysone and creates an autoregulatory loop that causes increases in the level of the receptor in response to increases of the hormone. EcR is the portion of the heterodimer that binds the hormone. The binding of the hormone-receptor complex at the early puff gene sites activates them and also represses the formation of late genes. The late genes are activated by products of the early genes that remove the repression that is induced by the ecdysteroid-receptor complex. The early gene products also repress the activity of the early genes themselves. The genes associated with the early puffs are thus regulators of late gene expression and the late genes consequently play a direct role in salivary gland morphogenesis. Many of these genes have been identified as encoding transcription factors.

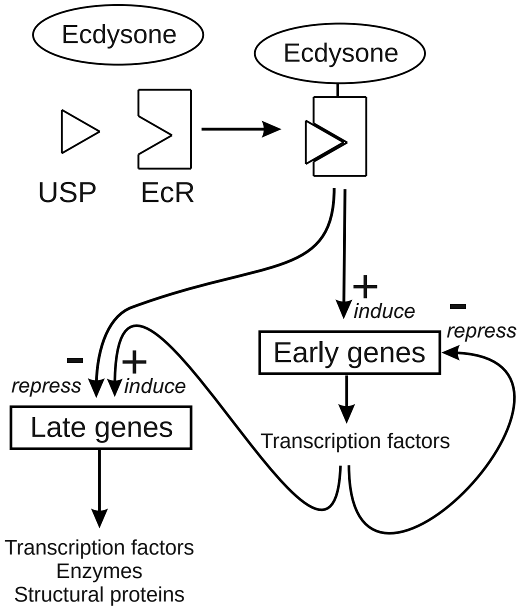


FIGURE 1.26. A model originally proposed by Ashburner (1974) for the action of ecdysteroids in the *Drosophila* salivary gland. The ecdysteroid receptor (EcR) and the product of the ultraspiracle gene (USP) bind to the hormone. The ecdysteroid receptor complex binds to early genes, stimulating their transcription but inhibiting the transcription of the late genes. The early gene product that is produced subsequently inhibits the early genes but stimulates the late genes, demonstrating the cascade of gene activity that is involved in salivary gland morphogenesis.

Although all tissues of the insect can potentially be exposed to the ecdysteroids that are circulating in the blood, not all cells respond in the same way. The temporal expression of specific isoforms of the EcR ecdysteroid receptor that may be present in a cell accounts for the ability of these certain cells to respond to the hormone. Different EcR isoforms have been identified in different cells that show different responses to ecdysteroid during metamorphosis. For example, the isoforms EcR-A, EcR-B1, and ECR-B2, which differ in their N-terminal sequences, are expressed in varying amounts in tissues that show altered responses during metamorphosis, such as imaginal discs and neurons destined to be remodeled in going from the larval to the adult stages. In *Drosophila* larvae, the EcR-B1 isoform is predominant in larval epidermal cells that are programmed to die during metamorphosis, whereas the EcR-B2 isoform is present in imaginal discs that proliferate and differentiate during metamorphosis. EcR-A is expressed in developing adult tissues and also in a group of neurons that die after adult eclosion when ecdysone levels decline. USP, which is a vertebrate retinoid X receptor homolog, also is produced in at least two isoforms. Once bound to ecdysone,

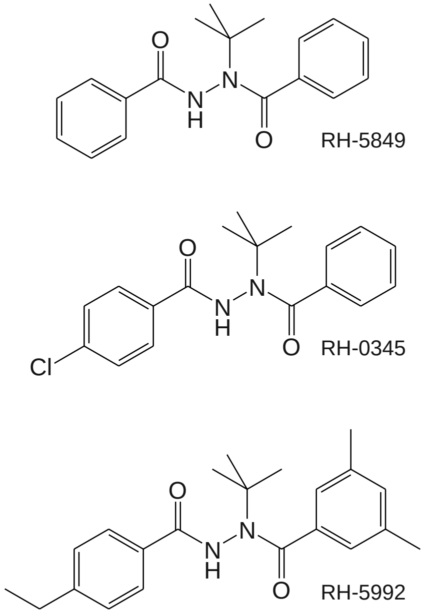


FIGURE 1.27. Nonsteroidal ecdysone agonists.

the affinity of the USP-EcR heterodimer to an ecdysone response element increases, and it then binds to the promoter region of specific genes to activate or inactivate their expression. This gene expression can be outwardly observed as a pattern of puffs. These differences in the type of DNA binding protein found in different cells may also be responsible for the varied sensitivity and responses of tissues to the hormone.

The identification of non-steroidal agonists, such as the substituted hydrazines RH-5849, RH-5992 or tebufenozide, and RH 0345 or halofenozide, have provided additional insights into the action of ecdysteroids and have promise for insect control (Figure 1.27). These agonists bind to ecdysteroid receptors and elicit the biological effects of the true hormone. Other antiecdysteroids such as certain plant brassinosteroids can compete with ecdysteroids for their receptor binding sites and block their action.

THE JUVENILE HORMONES

Juvenile hormone (JH) was first described by **Wigglesworth** as an "inhibitory hormone" that prevented the metamorphosis of the blood-sucking hemipteran *Rhodnius prolixus*. *Rhodnius* has five larval instars, each of which will molt after

it ingests a large meal of blood. When an active corpus allatum, the source of JH, was implanted into last instar larvae, the recipients of the gland molted to supernumerary larvae, or additional larval instars, after they fed rather than producing the adult cuticle that would normally be expected. Also, if last instar larvae were connected to early instar larvae by parabiosis, in which the circulatory systems of the two insects intermingled, the more mature member of the pair continued to express larval characters after it molted. This indicated that a factor circulating in the blood of the younger member was responsible for the retention of larval characters in the older member of the pair. Wigglesworth coined its present name of juvenile hormone when it became clear that the hormone acted to promote the expression of larval characteristics rather than to inhibit adult ones. Juvenile hormone is now the generic name for several sesquiterpenes that mediate a wide variety of functions in addition to metamorphosis. Similar to ecdysteroids, JH has multiple effects during the life of an insect, and its specific involvement in the processes of metamorphosis, diapause, reproduction, and metabolism will be described in later chapters. In fact, the name "juvenile hormone" is certainly a misnomer considering the versatility of the hormones within this group.

Major Types of JH and Their Synthesis

The identification of JH became possible with the serendipitous discovery of large quantities of the hormone. While **Williams** was performing an experiment designed to extend the life of male *Hyalophora cecropia* by parabiosing them to pupae, he noted that the parabiosed pupae molted to other pupae rather than to adults. This suggested to him that the male was supplying JH, and its accessory glands indeed contained large amounts of the hormone. With this plentiful supply of JH, sufficient quantities were finally available for its analysis.

Six major members of the juvenile hormone group are currently recognized (Figure 1.28). The first structural identification from the male accessory gland material showed the hormone to be a sesquiterpenoid epoxide with an 18-carbon skeleton. After a second JH was subsequently identified from *H. cecropia* in smaller amounts, the two hormones were named **JH I** and **JH II**. As a lower homologue, the 17-carbon JH II contained a methyl group at carbon 7 instead of the ethyl group characteristic of JH I. A third homolog, **JH III**, was identified from the corpora allata of *Manduca sexta* cultured *in vitro*. This 16-carbon homolog contained three methyl groups and for many years was the only JH found in insect orders other than Lepidoptera. As the simplest of the juvenile hormones, it may represent the structure from which the others are derived. A fourth JH was isolated from the developing eggs of *M. sexta,* along with smaller quantities of JH I, and as the next higher 19-carbon homolog of JH I, it was named **JH 0** according to the convention that developed of naming higher

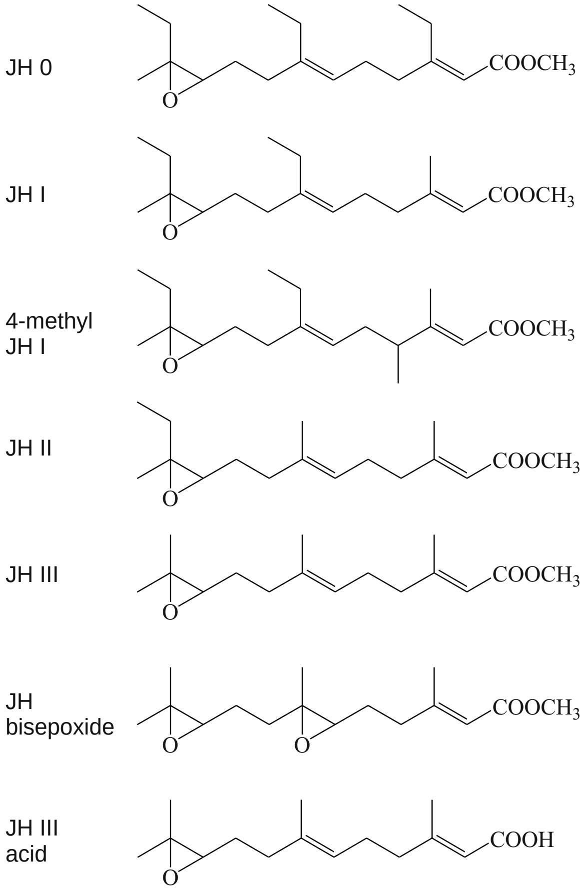


FIGURE 1.28. Some of the major juvenile hormones that have been identified in insects.

homologs with lower hormone numbers. A fifth JH, another 19-carbon homolog, was identified in developing embryos of *M. sexta* as **4-methyl-JH I**.

A recently identified JH was recovered from cultured larval ring glands of *Drosophila melanogaster*. This **JH III bisepoxide** contains a second epoxide group and has been found in several dipterans as well as in ticks. **JH acids**, natural degradation products of JH metabolism, are also produced by the corpus allatum of *Manduca* larvae and may serve as a hormone in their own right, because the imaginal discs may be capable of the acid methylation necessary to activate them. Several **hydroxy juvenile hormones** are produced by the CA of locusts and cockroaches (Figure 1.29). This hydroxylation may result in molecules with greater biological activity, just as 20-hydroxyecdysone is more active than ecdysone.

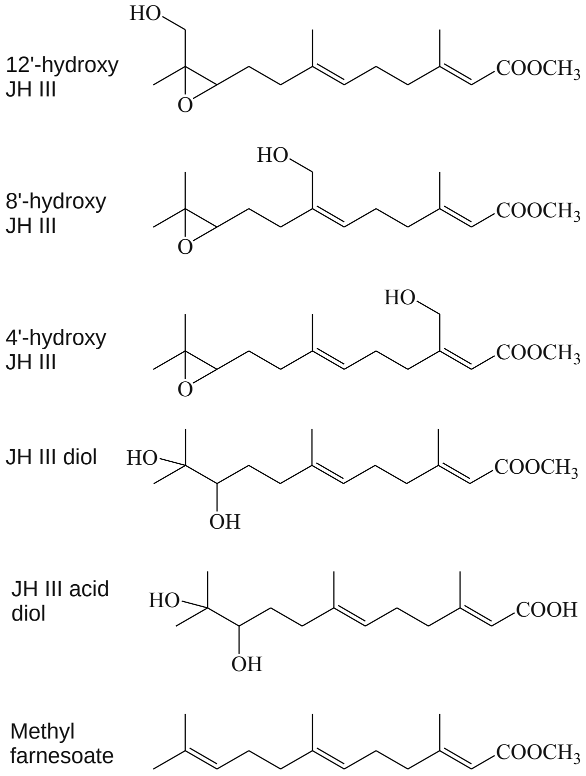


FIGURE 1.29. Other natural juvenile hormones.

In a similar manner as for the ecdysteroids, some plants produce molecules with JH activity. These **phytojuvenoids** include the actual insect hormones JH III and farnesol produced by the Malaysian sedge. A serendipitous discovery during the course of failures in rearing the bug *Pyrrhocoris apterus* led to the identification of the **juvabiones** (Figure 1.30) when a factor in the paper used to line the cages of the insects was traced to the Canadian balsam fir. Although initially considered as a general JH mimic, juvabiones were found to be active only against these pyrrhocorid bugs. **Juvocimeme-2** was isolated from the oil of sweet basil and showed activity against milkweed bugs. A survey of plants for JH and anti-JH activity by **Bowers** yielded the **precocenes** (Figure 1.31) that destroy the cells of the CA and prevent them from synthesizing JH. These are effective against several hemimetabolous species but not holometabolous ones, and they are fairly toxic to vertebrates, factors that make them unsuitable control agents.

We can only speculate about the possible reasons for so many different JH homologs. They may have been originally involved in reproduction in ancient

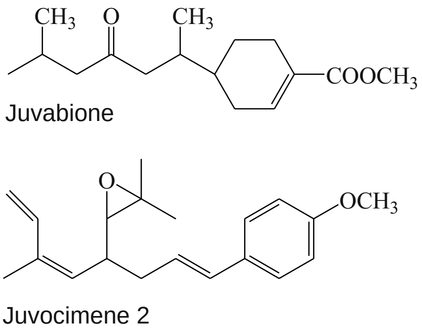


FIGURE 1.30. Juvabiones produced by plants.

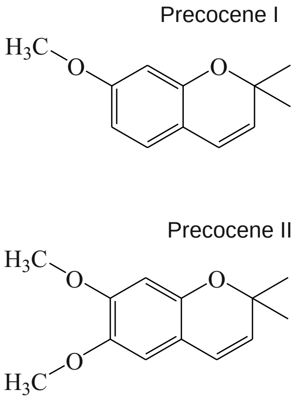


FIGURE 1.31. The precocenes that attack the CA and prevent JH synthesis.

insects, but their roles expanded evolutionarily to include metamorphosis, diapause, behavior, and caste determination. JH is present in all insect orders, including the primitive Collembola that are considered to be outside and ancestral to the insects, thus probably utilized by insect ancestors for 400 million years. The time at which JH was programmed to appear during development may have been the key to the evolution of the larval and pupal stages of holometabolous insects. Farnesoic acid and methyl farnesoate are used in reproduction and as a juvenilizing agent in Crustacea and also are products of the CA in several cockroaches. Advanced insects generally use the higher homologs of JH 0, I, and II, iso-JH 0, methyl JH I, and the JH bisepoxide. In Lepidoptera and higher Diptera, multiple JH forms may be present in the same insect. There are differing biological activities among the homologs; JH I and II tend to be more active in morphogenesis than does JH III.

The JH III homolog is found in most insect orders, including those considered to be the most primitive, and the pathway to its synthesis is consequently believed to also be the most primitive. JH III is synthesized from three molecules of acetate to form mevalonic acid that leads to the isoprenoid building blocks of isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAP). Two molecules of IPP and one of DMAP are assembled to form farnesyl pyrophosphate that is ultimately converted into JH III (Figure 1.32). The formation of the side chains in the higher homologs JH I and II involve the combination of propionate with acetate to give rise to homoisopentyl pyrophosphate (HIPP) and ethylmethylallyl pyrophosphate (EMAP). The condensation of two IPP and one EMAP form JH II, and one EMAP, one HIPP, and one IPP form JH I. JH 0 originates from one EMAP and two HIPP (Figure 1.32). More detailed steps in the synthesis of JH III are shown in Figure 1.33. Higher JH homologs result from the incorporation of homomevalonate instead of mevalonate.

Common to all juvenile hormones are several functional groups necessary for their morphogenetic activity. Pathways of biological degradation involve the loss or alteration of these groups. The inactive JH acid results from the loss of the methyl ester in the C-1 position. JH acid is produced at specific times by the CA of some insects and also may be a prohormone. The inactive **JH diol** is

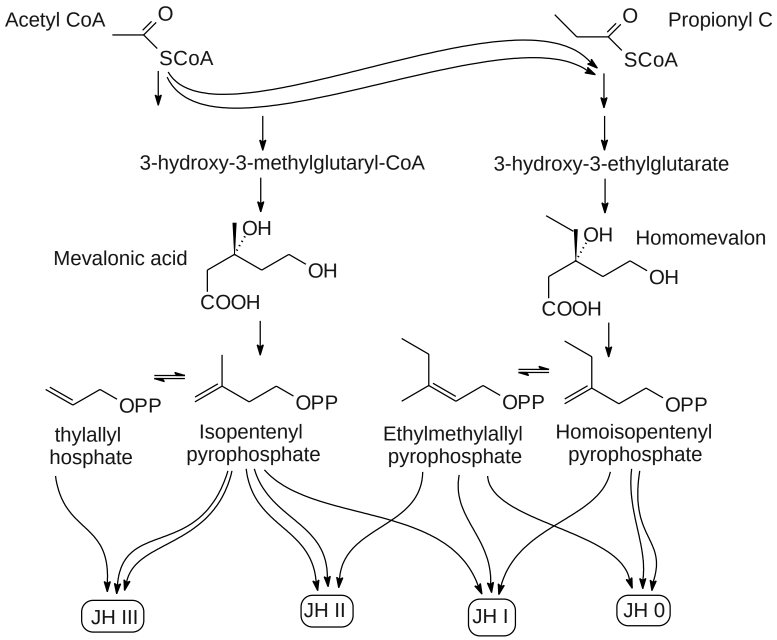


FIGURE 1.32. Initial steps in the synthesis of common juvenile hormones.

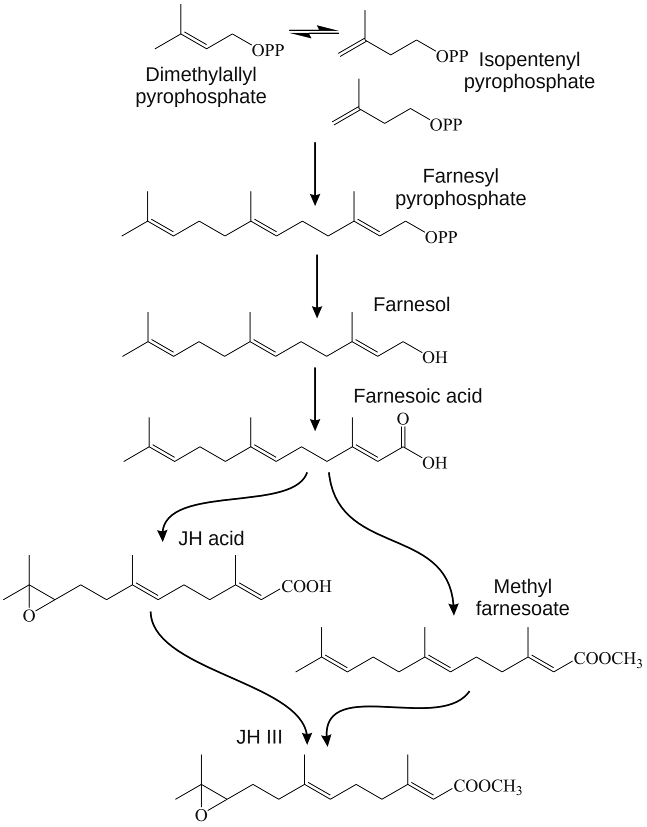


FIGURE 1.33. Final steps in the synthesis of JH III.

produced by the loss of the epoxide in the C-10,11 position. The three unsaturated positions also modulate biological activity; saturation of the 2-ene double bond drastically reduces activity, while the saturation of the 6-ene double bond can increase activity. Loss of the methyl branches along the chain reduces biological activity. An isoprenoid chain length of at least 10 —CH_2— units appears to be necessary for biological activity, with 14 to 16 being optimal. Given the importance of JH to so many physiological processes in insects and its inactivity in vertebrates, fish, and many aquatic crustaceans, several JH analogs have been developed and proposed for use in insect control. An understanding of how modifications of the JH molecule affect activity is necessary to develop additional synthetic analogs that may be even more effective. The native isoprenoid juvenile hormones are unstable and unsuitable for use, but the analogs that have been synthesized show sufficient stability yet are not overly persistent in the environment. The structures of some JH analogs that have been used in insect management programs are shown in Figure 1.34.

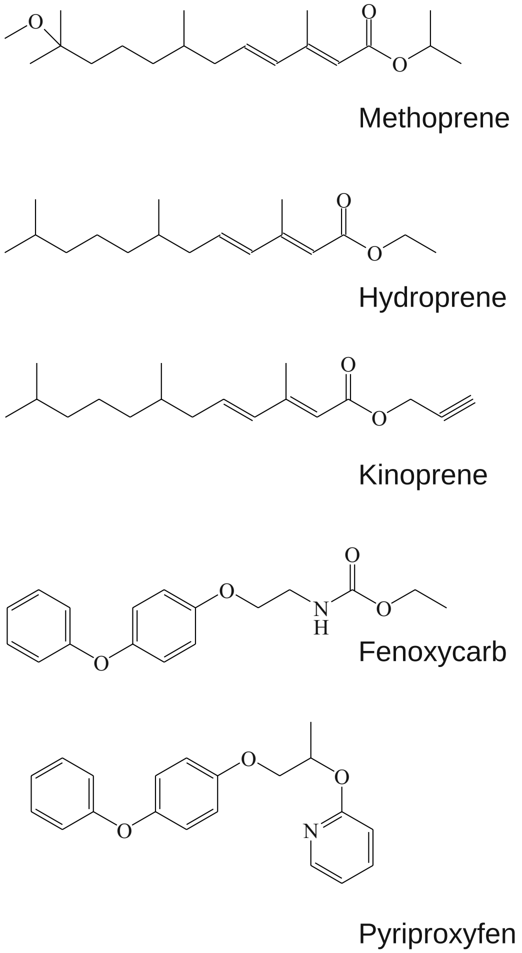


FIGURE 1.34. Examples of some JH analogues used in insect control.

Sites of JH Synthesis and Control of JH Production

The corpus allatum (CA) is the major organ of JH synthesis and release, although other tissues such as the male accessory glands and imaginal discs are able to convert JH acids to JH but are unable to synthesize the basic carbon skeleton. The CA is ectodermal in origin and is usually located in the posterior regions of the head. More primitive insect orders contain a paired CA located ventrally, but in more specialized insect orders, it has migrated to a more dorsal location. In the higher Diptera (suborders Brachycera and Cyclorrapha), the pair is fused into one structure and is located dorsal to the aorta; in the Embioptera,

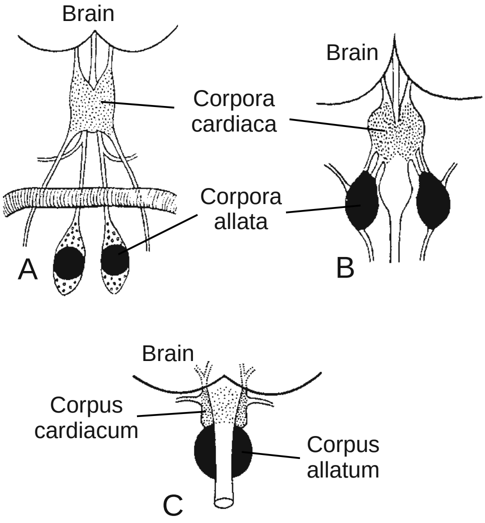


FIGURE 1.35. The location and structure of the corpus allatum in (A) a mosquito, (B) a cockroach, and (C) a hemipteran. From Cymborowski (1992). Reprinted with permission.

Hemiptera, Dermaptera, and Pscoptera, they are fused ventral to the aorta (Figure 1.35). The CA can contain both intrinsic glandular cells as well as neurosecretory cells that have their origins elsewhere. They are generally innervated from the brain by two pairs of nerves, the nervi corporis allati I (NCA I) that originate in the brain and pass through the corpus cardiacum (CC) on their way to the CA and the NCA II that originate in the subesophageal ganglion. Three other nerves, the nervi corporis cardiaci I, II, and III (NCC I, II, and III), originate in the brain and enter the CC and may also influence the CA (Figure 1.15).

Given the central role of the CA in development and reproduction, sophisticated and precise mechanisms of activation and inhibition have evolved to assure both the timely production as well as the cessation and clearance of JH. Hemolymph JH titers are regulated by both biosynthesis and degradation. The most important factor that determines JH titer is its synthesis by the CA. Because JH is not stored in the CA, its release is dependent on synthesis, and this synthesis is rigidly controlled along several avenues. Environmental stimuli, such as photoperiod, and endogenous factors, including mating and nutritional state, are integrated by the brain, and CA activity is then regulated both neurally and through the release of neurosecretory hormones, with each possibly having stimulatory or inhibitory effects. For example, in the last larval instar of most insects, JH production ceases either as a result of the absence of neural stimulation or the presence of neural inhibition from the brain. This neural control is generally affected by the NCA I; although the NCA II innervates the CA

from the subesophageal ganglion, these connections do not appear to be as important.

Neurohormones have also been identified that affect CA activity. These were initially suspected because of the stimulatory effect of brain extracts on JH production *in vitro*. These substances called **allatotropins** stimulate JH production. Allatotropins have been associated with positive CA regulation in both larvae and adults. They may originate in the neurosecretory cells of the brain, but they have also been found in the frontal ganglion and the terminal abdominal ganglion. The first allatotropin to be isolated and sequenced came from *Manduca sexta* and is now called **Manse-AT**. It is a 13 amino acid peptide whose gene encodes three prohormones that differ from each other by alternative splicing and that may allow the peptide to serve different roles during different insect life stages. There appears to be little variation in the structure of the allatotropins among insect species; Manse-AT is also present and functional in other lepidopterans and some dipterans. It enhances JH production in honey bees and flies but is not active in *Tenebrio* beetles or *Schistocerca* locusts. Manse-AT is suspected to have other functions, including the stimulation of gut motility, midgut ion transport, and even the entrainment to circadian rhythms. A second, unique allatotropin, Aedes-AT, has been identified from the abdominal ganglia of mosquitoes, and at least seven other allatotropins that have not yet been identified are suspected to exist in other insects. Some peptides that are transferred to the female during mating that originate in the male accessory glands are also capable of regulating JH synthesis.

In contrast, there are other neuropeptides produced by the brain called **allatostatins** that inhibit JH synthesis by the CA. They were originally isolated from the brain of the cockroach, *Diploptera punctata*, and more than 150 related allatostatins have since been isolated from moths, flies, and other cockroaches. They are grouped into three larger families (A, B, and C) based on shared amino acid sequences and their presence in various insect orders. The *Manduca sexta* allatostatin, **Manse-AST**, placed within the allostatin C family, is composed of 15 amino acids, is structurally unrelated to those from cockroaches, and has no effect on the cockroach CA. Peptides similar to the cockroach allatostatins have been isolated from dipterans, but these do not appear to have any allatostatic effects in those species. Instead, they may have other biological functions including the modulation of muscle contraction and gut motility and the inhibition of vitellogenin release from the fat body. The oviducts of the female reproductive system, the antennal pulsatile organ, and midgut ion transport are affected by these peptides. It may be that the peptides were originally myomodulatory and assumed a secondary role of CA regulation in the cockroaches and their relatives. The true allatostatins are either delivered directly to the CA by axons of the lateral neurosecretory cells of the brain or through the hemolymph and regulate the early steps of JH synthesis involving the formation of acetyl CoA. The developmental timing of allatostatin receptors in CA cells largely determines the

effect of the peptide on JH synthesis. An **allatoinhibin** has been identified from the brain of *Manduca* that inhibits the CA nonreversibly, unlike the reversible action of allatostatins. Little is known about this factor and it has not been reported to occur in any other species.

In addition to substances from the brain, the activity of the CA in female cockroaches is affected by a humoral factor from the ovaries. The removal of the ovaries reduces JH synthesis and their reimplantation restores it. Ecdysteroid receptors have been identified in the CA of *Manduca*, suggesting that JH production may also be regulated by ecdysteroids. Increases in ecdysteroid concentrations associated with pupal commitment can increase levels of JH acid that are necessary for pupal development. The physiological state of the CA itself may also determine its sensitivity to these various neuropeptides, as the way that the CA responds to allatostatins may change during the reproductive cycle of the female. Finally, high levels of JH itself may suppress CA activity through feedback regulation.

The overall regulation of the CA, integrating the effects of neural and humoral pathways, is shown in Figure 1.36. The capacity of the CA to produce JH is determined by stimulatory and inhibitory signals that arrive both through the hemolymph and nervous system. The brain can stimulate and inhibit the CA by the nerves that innervate it and produce neuropeptides that function in a similar manner. In the cockroach, allatostatins are delivered to the CA by lateral neurosecretory cells that leave the brain via the NCCII. Thus, we see that like the ecdysteroids, an important hormone like JH has a precise mechanism of control to assure that its proper physiological concentrations are maintained when required at times that are biologically appropriate.

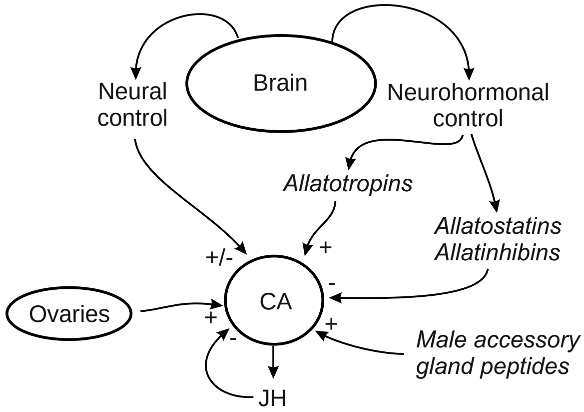


FIGURE 1.36. A summary of the ways that the corpus allatum can be regulated. The brain can stimulate or inhibit juvenile hormone synthesis by nervous or neurohormonal signals. The allatotropins stimulate JH synthesis; the allatostatins and allatinhibins inhibit it. There is some evidence for feedback regulation by circulating JH and also stimulation by the ovaries.

Postproduction Regulation of JH Activity

Once secreted by the CA, JH is transported to target tissues through the hemolymph, but because of its lipophilic nature, JH must first bind to other molecules in order to move through the aqueous medium. Perhaps more important, binding can also protect the hormone from degradation by nonspecific tissue-bound esterases. There are a variety of such molecules that bind JH for transport and protection, collectively termed the **juvenile hormone binding proteins** (JHBP). Most of the circulating JH is bound to these JHBPs, leaving little JH in free circulation. They not only move the lipophilic JH throughout the hemolymph, but they also may be storage sites for hemolymph JH.

Three types of these binding proteins have been described in insects. The **high affinity, low molecular weight binding proteins** are synthesized by the fat body and consist of a single polypeptide that contains one JH binding site. Upon JH binding, the protein undergoes a conformational change and protects the hormone from ester hydrolysis. Another group of **high affinity, high molecular weight binding proteins** are also known as **lipophorins** that shuttle many different lipids between tissues within the insect. Lipophorin is composed of two apoproteins of 230–250 and 70–80 kDa and has multiple JH binding sites. The third type of binding protein found at lower concentrations than the others is generically known as **hexamerin**, composed of six subunits and multiple JH binding sites.

The onset of metamorphosis following the last larval instar requires the clearance of JH from the hemolymph in order for epidermal cells to switch their commitment in the presence of ecdysteroid to the developmental pathways that lead to the production of pupal and adult cuticles. Although JH titers are reduced when the CA ceases its synthesis, the reduction of circulating JH titers is accomplished by degrading the JH that has already been produced. There are two major routes by which this degradation of JH occurs (Figure 1.37). Specific JH esterases (JHE) recognize the JH-JHBP complex and hydrolyze the methyl ester of JH to produce the JH acid. When interacting with the JH-JHBP, JHE may institute a conformational change in the JHBP, causing the JH to be released from its binding pocket. JH epoxide hydrolase hydrates the epoxide to produce the JH diol, but its ability to degrade JH is reduced when JH is bound to the JHBP and it may be more effective against unbound JH or JH acid. High levels of JHE are correlated with low JH titers, clearing the hemolymph of any residual JH activity once the CA stops production. General esterases are also present that are able to hydrolyze both JH and other general substrates, but these are probably less important in the regulation of JH levels than are the specific esterases.

Several chemicals that act as JH esterase inhibitors have also been developed and used to study JH catabolism, but they have not seen commercial use for control because when applied topically, their activity is relatively short lived. These inhibitors include the trifluoromethylketones, which bind JH

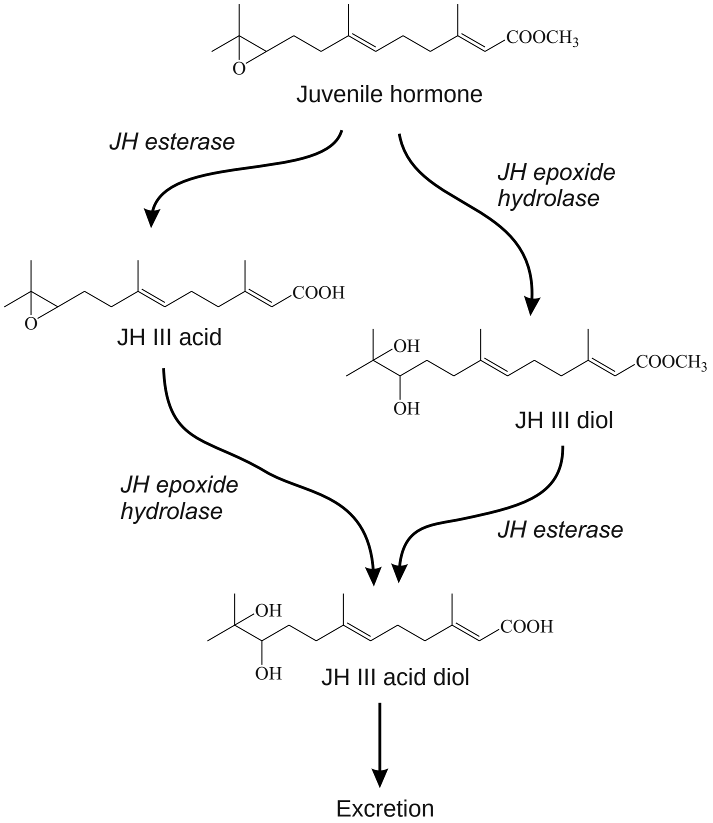
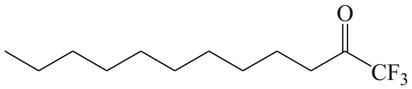


FIGURE 1.37. Degradation of JH by JH esterase and JH epoxide hydrolase. The acid diol is formed and excreted by the initial formation of either the JH acid or the JH diol.

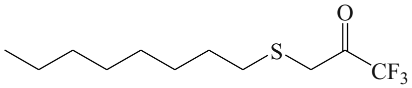
esterase reversibly, and the phosphoramidothiolates, which bind irreversibly (Figure 1.38).

Mode of Action of JH

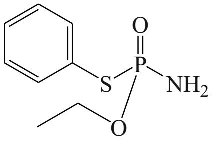
The major role of JH in insects is to modify the action of ecdysteroids and prevent the switch in the commitment of epidermal cells. In the presence of ecdysteroids, JH preserves the current program of gene expression. JH both influences the stage-specific expression of the genome that is initiated by ecdysteroids and also acts by itself to modulate the expression of certain specific genes. The search for the specific nuclear JH receptor that binds the hormone has been elusive, although there is strong evidence that in *Drosophila*, JH III binds to USP,



TFT
1,1,1-trifluorotetradecan-2-one



OTFP
3-octylthio-1,1,1-trifluoro-2-propanone



EPPAT
O-ethyl *S*-phenyl phosphoramidothiolate

FIGURE 1.38. Representative JH esterase inhibitors. TFT and OTFP are trifluoromethylketones, and EPPAT is a phosphoamidothiolate.

one of the components of the heterodimer that also binds ecdysteroids. The hormone induces a different conformational state when bound to USP, with the heterodimer then inducing transcription of specific genes. The relationship of both JH and ecdysteroid binding to different components of the heterodimer explains how JH might modulate the effect of the cellular response to ecdysteroids.

There is some direct evidence for the existence of JH-induced transcription factors that bind to the response elements of JH-induced genes. In the locust, *Locusta migratoria*, a JH response element, a short sequence of DNA located in the promoter of hormone-responsive genes, has been identified, along with a JH-induced protein, tfp1, that binds to it as a transcription factor. The evidence suggests that JH induces an association of tfp1 with other proteins to form an active transcription factor that binds to the JH response elements in the promoters of JH-induced genes.

JH also induces the expression of the ecdysone-induced nuclear receptor E75A, and when bound to E75A, JH represses the activation of a group of early genes including those for the **Broad** (BR) transcription factor, a product of the *broad* gene; *broad* is one of the early genes that is directly transcribed when exposed to ecdysteroid and is also regulated by JH. BR expression is associated with pupal commitment and the late genes that are expressed during this time.

During the final instar of holometabolous larvae, JH titers are reduced and the small pulse of ecdysteroid initiates metamorphosis. This metamorphic switch

involves the inactivation of larval specific cuticle genes and the new transcription of pupal cuticle genes. The specification of pupal cuticle genes by the epidermal cells results from the expression of the BR transcription factor. A high level of BR RNA is expressed when the final larval instar becomes committed to the pupal instar, but only when JH is absent; JH application prevents both BR expression and pupal commitment. Other early genes, including *E74* and *E75*, also encode a set of transcriptional regulators that activate a cascade of late genes that are involved in metamorphic processes such as pupal cuticle formation, proliferation, and differentiation of imaginal disc cells, and the programmed death of other cells no longer required in the pupal stage. The nuclear receptor E75 also contains a heme prosthetic group that binds to carbon monoxide and nitric oxide and may function as a gas sensor. Expression of the *broad* gene and its six isoforms is key to the successful progression through the steps of metamorphosis. The combinations of the isoforms that are expressed in each tissue determine the stage-specific response to ecdysteroids.

A simple model only begins to describe the very complex system. For a larval-to-larval molt, both 20HE and JH are present and stimulate the production of the nuclear receptor E75A (Figure 1.39A). This receptor is responsible for the activation of several JH-inducible genes that are involved with larval growth, but it represses BR and its own expression. Once larval development is complete, 20HE in the absence of JH activates another group of early genes — *BR*, *E74*, and *E75* — that in turn activate a set of late genes responsible for pupal metamorphosis (Figure 1.39B). The crosstalk between ecdysone and JH is possible by using *E75* as a common element in both signaling pathways.

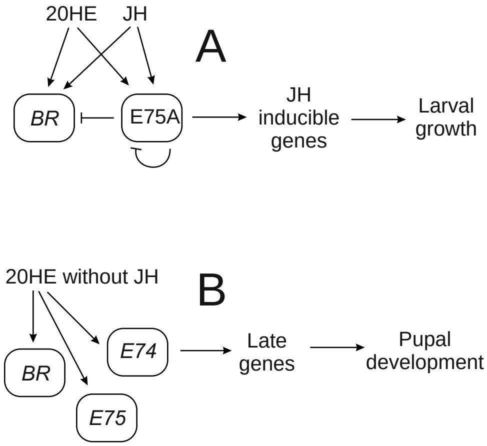


FIGURE 1.39. Model for the role of Broad in (A) larval and (B) pupal development.

OTHER NEUROPEPTIDES FOUND IN INSECTS

The existence of ecdysteroids, juvenile hormones, and PTTH have been known since the beginnings of insect physiology and are considered to be major hormones that regulate development and reproduction in insects, but there are many other more recently discovered hormones that are equally as important. **Adipokinetic hormone** (**AKH**) is both synthesized and released from intrinsic neurosecretory cells of the corpus cardiacum. AKH is actually a family of related peptides with varied functions in insects. The most important and well-studied function of AKH is in the regulation of lipid recruitment for flight. AKH is released in response to stimuli from prolonged flight and acts on the fat body and flight muscles to cause the mobilization and utilization of diglycerides once hemolymph carbohydrates are exhausted (see Chapter 6). **Diuretic neuropeptides** are involved in the maintenance of water balance and as clearance factors that increase the filtration rate of the hemolymph. Diuretic peptides generally act by increasing the activity of the Malpighian tubules and inhibiting the resorption of water from the hindgut. Most diuretic neurohormones have a varying degree of homology with the corticotropin-releasing factor of vertebrates, but in the blood-sucking hemipteran, *Rhodnius prolixus*, the biogenic amine **serotonin** serves as a diuretic hormone (see Chapter 8). **Proctolin** is a small pentapeptide that causes contractions of the longitudinal muscles of the proctodeum of the hindgut. It appears to be a neuromodulator that works together with the neurotransmitter glutamate at the neuromuscular junction. Proctolin, along with several members of the AKH family, is a cardioacceleratory hormone that also affects the rate of the heartbeat. A large number of other myotropic peptides, such as the **leucokinins** and **acheatakinins**, have been identified in insects. **Pheromone biosynthesis activating neuropeptides (PBAN)** are synthesized in female lepidopterans that produce sex pheromones to attract males. PBAN regulates the enzymes that lead to pheromone biosynthesis (see Chapter 12). **Eclosion hormone (EH)** and **crustacean cardioactive peptide (CCAP)** release the behaviors programmed into the CNS that express the stereotypical sequence of peristalsis and other movements associated with eclosion. They are activated by an **ecdysis-triggering hormone (ETH)** produced by **Inka cells** that are attached to tracheae near the spiracles (see Chapter 2). CCAP, originally isolated from the shore crab, also triggers the release of AKH from the corpora cardiaca of locusts. A **diapause hormone** produced by the *Bombyx mori* female while she develops her eggs regulates their entry into an embryonic diapause. **Bursicon** is a neurosecretory hormone that mediates the sclerotization of the cuticle immediately after it is synthesized after the molt. It may also be involved in the wound repair of the epidermis (see Chapter 2).

A large number of insect neuropeptides have C-terminal sequences that are similar to FMRFa, a cardioexcitatory peptide isolated from mollusks named after its amino acid sequence (Phe-Met-Arg-Phe-amide). These peptides play

important roles as neurohormomes and neuromodulators of muscle activity in insects.

VERTEBRATE-TYPE HORMONES IN INSECTS

Insects were once believed to be devoid of any hormones and even lack brains, and it is still surprising to learn that so many life processes of vertebrates can also be found in insects. Of course, because insects are the more ancient group, it is probably more correct to refer to the insect-type hormones that are present in vertebrates. The operation of such essential systems as protein synthesis, muscle contraction, and cell metabolism do not differ significantly between us and insects. An indication of the unity that exists between insects and vertebrates is best described by the example of the female rabbit flea that depends on the hormones circulating in the blood of its pregnant female vertebrate host in order to reproduce. It should therefore not come as a surprise that many hormones previously isolated from vertebrates are also present in insects, although their parallel functions have yet to be fully determined, and important differences in amino acid structure question their true homologies with vertebrate peptides.

Insulin controls the rate of glucose transport across cell membranes and relays nutritional information that organisms use to regulate their growth. Its functions involving the regulation of growth have been largely conserved in insects. Bombyxin, the small PTTH, shares a significant sequence homology with the A chain of vertebrate insulin. In *Bombyx mori*, the melanization-reddish coloration hormone shares some sequence homologies with insulin-like growth factor II. **Gastrin** and **cholecystokinin (CCK)** are related peptides that respectively mediate an increase in the secretion of acid in the vertebrate stomach and cause the gallbladder to contract. In insects, the **sulfakinins** show structural and functional similarities to gastrin and CCK. **Somatostatin** is a master hormone in vertebrates that controls the release of many other hormones. Somatostatin-like peptides have been found in such diverse insects as crickets, hoverflies, and locusts. Adipokinetic hormone regulates lipid mobilization from the fat body in insects and has homologies with vertebrate **glucagon**. Insect **tachykinins** stimulate visceral muscles and are structurally homologous with vertebrate tachykinins that are involved in processes as diverse as salt balance, sensory processing, and gut motility. **FMRFamide-related peptides** are found in many vertebrates and invertebrates. They have been isolated from insects where they stimulate muscle contraction and frequency of heartbeat, and they regulate behavior in female mosquitoes. **Melatonin** is produced by the vertebrate pineal gland during the scotophase and causes drowsiness in humans. Its occurrence in the compound eyes of locusts and in a variety of other insects suggests it may also be involved in photoperiodism in invertebrates. Melatonin levels in several insects show a circadian rhythmicity, and the hormone has been implicated in the circadian

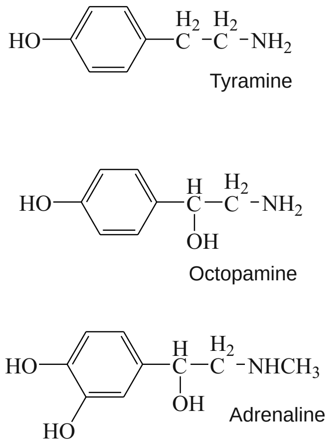


FIGURE 1.40. Biogenic amines. Tyramine and octopamine have only been found in invertebrates.

release of PTTH in cockroaches. **Octopamine** and **tyramine** are the invertebrate counterparts to the adrenaline and noradrenaline of vertebrates (Figure 1.40). They are classified as biogenic amines and are the only nonpeptide hormones found exclusively in invertebrates. Functioning variously as neurohormones, neurotransmitters, and neuromodulators, they regulate an abundance of physiological processes in insects, including the behaviors involved in the flight-or-fight response, energy metabolism, learning and memory in bees, muscle contraction, and the sensitivity of sensory neurons.

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CHAPTER 2

Integumentary Systems

The components that constitute the exoskeleton make an overwhelming contribution to the terrestrial success that arthropods can claim. Like the skin of vertebrates, the exoskeleton completely covers the insect and additionally provides an armor-like protective suit as a skeleton. Its most critical function is to serve as an interface between the insect and the environment, providing a barrier for the movement of water, ions, parasites, and environmental chemicals including insecticides. This barrier is especially significant for small animals like insects that have a high surface-to-volume ratio and therefore present a relatively large amount of surface area to the environment. The nature of the exoskeleton has thus had profound implications for growth, respiration, locomotion, and, from a human perspective, the design of chemicals that must penetrate the integument to be used as control agents.

The exoskeleton also plays an important structural role in determining the form of the insect body and making possible the dramatic changes in form that accompany metamorphosis. Insects exploit a variety of diverse habitats and diets, made possible by a developmental plasticity in body form and mouthpart structure. The rigidity that it provides allows for the insertion of muscles that can produce more precise locomotor movements than can the soft hydrostatic exoskeletons of the annelid worms. Although being surrounded by a rigid suit of

armor might limit the movement and environmental awareness of insects, the integument that makes up the exoskeleton is elastic in some areas to make flying and walking possible. Numerous sensory receptors that are concentrated in strategic areas provide windows to the outside world that allow the insect to respond appropriately to the environment. The terms "integument" and "exoskeleton" are often used interchangeably, depending on which functional component is under consideration.

The integument may comprise up to half the dry weight of some insects, representing a major investment of raw materials. However, because much of this is resorbed during molting and even periods of starvation, the integument could also be viewed as a food reserve. The hydrocarbons that are deposited in the exoskeleton are responsible for releasing particular behavioral sequences that are involved in mating. Specific structures required for mate recognition, as well as chemicals such as pheromones and pigments that are deposited in the exoskeleton, are releasers for the stereotyped behaviors that are necessary for mating to occur. All of these features are provided by a single layer of epidermal cells and their secretions.

For an animal the size of an insect, an exoskeleton provides significant mechanical advantages over an endoskeleton of the same weight. Given two appendages that are of identical skeletal cross-sectional areas, the one with an exoskeleton is three times stronger than the one with an endoskeleton because of the way that the margins of the appendage, and not the center, bear the most stress when it is bent. To be of equivalent strength, the endoskeletal appendage would have to occupy over 80% of the total appendage cross section and would contribute significantly to the weight of the animal and its investment in structural materials. Using a building analogy, the exoskeleton is more similar to the lightweight scaffolding that surrounds a structure during its construction than it is to the endoskeletal girders that are used to permanently create it.

Although the exoskeleton provides a number of advantages, it does pose a major problem for growth. For insects with rigid exoskeletons to undergo significant amounts of growth, a new, larger exoskeleton must first be synthesized and the older one discarded. During this period of molting, the insect is relatively helpless against predators because flight or defense is difficult. Molting consumes time, energy, and metabolic resources. There is also a potential susceptibility for the loss of water because the insect can neither drink nor adjust its body to a changing environment. To reduce this susceptible period during the molting cycle, more advanced insects have evolved toward a reduced number of molts. Growth during the intermolt period is possible because the larvae of advanced holometabolous insects generally have relatively unsclerotized cuticles that can undergo a degree of stretching.

INSECT GROWTH AND DEVELOPMENT

The growth and development of insects is largely a function of the growth and development of their integuments. The cuticular molts that punctuate postembryonic growth are necessary if hard-bodied insects are to undergo any significant increases in size. Increases in body size do not always follow from molting, however. Insects that are starved during the larval stage or molt to a diapause form may actually molt to smaller individuals if they molt at all. Apterygote insects continue to molt into the adult stage, but pterygote insects are incapable of molting as adults. The inability of adult pterygote insects to molt is probably the result of the degeneration of the epidermal cells that produce the wing once it is formed. After the molt to the adult stage, the epidermal cells that make up the wing degenerate and the loss of the water contained within them makes it possible for the wing membranes to move rapidly for flight. However, these dead cells can no longer initiate a molt nor synthesize a new cuticle. Without living epidermal cells, another wing cuticle could not be formed if the insect molted again. Thus, the death of the cells that make flight possible also makes molting as an adult impossible. Only the winged mayfly subimago is capable of a molt to another winged form, but the subimago is a poor flier because living epidermal cells must remain as part of the wing.

In many holometabolous insects, considerable growth can occur during a single larval instar in the absence of a molt because, with the exception of the head capsule, the cuticle is extensible enough to accommodate some increases in size. The last instar of *Manduca sexta* can grow from 1 g to over 9 g in weight without a molt because the pleated outer epicuticle of the exoskeleton is able to stretch to accommodate the growth that occurs within this instar. However, a molt may ultimately be necessary in order to acquire a larger head capsule and allow the sclerotized mouthparts to increase in size so the rate of food intake is increased to satisfy the demands of the larger body.

STRATEGIES FOR GROWTH

The change that occurs as an insect develops from an immature to an adult is called "metamorphosis," literally a "change in form." A metamorphosis is considered as a partition in the life of an individual that separates an early feeding stage from a later reproductive stage. Insects show three major metamorphic strategies for reaching the adult stage, with the degree of metamorphosis dependent on the degree of divergence between the immatures and adults. **Ametabolous** development is the least advanced strategy and is found in the apterygote orders Thysanura and Archeognatha, which are primitively wingless insects (Figure 2.1). The approximately 8000 species that undergo this form of develop-

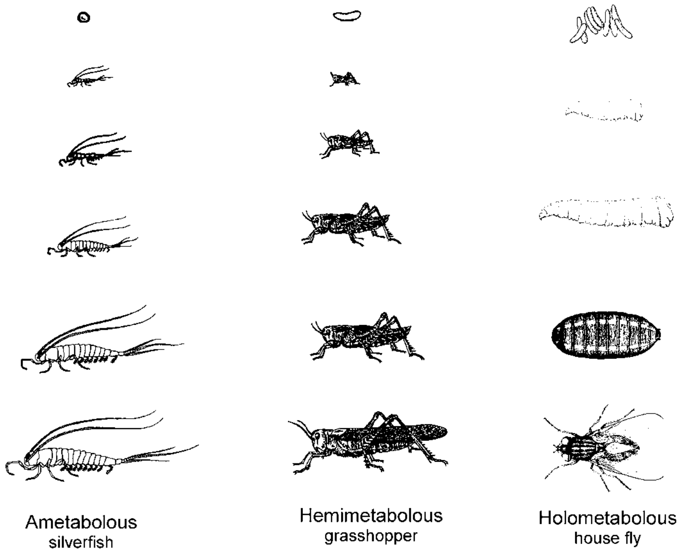

FIGURE 2.1. The three major types of metamorphosis in insects.

ment show little differences between immatures and adults except for their relative sizes and the presence of genitalia. Both the immatures and adults occupy the same habitats. They continue to molt once they become sexually mature adults, and the developmental strategy is considered to be the most primitive because there are few changes and consequently no real metamorphosis.

Those 100,000 or so insect species that engage in **hemimetabolous** development, or incomplete metamorphosis, also generally occupy similar niches as larvae and adults (Figure 2.1). Immatures lack wings and genitalia and the extent of their metamorphosis is the development of these structures in adults. Both have compound eyes, but those in adults may have more optical units, or **ommatidia**. Hemimetabolous larvae and adults may also show differences in cuticular structure. In hemimetabolous *Rhodnius prolixus*, numerous plaques distinguish the larval cuticle, whereas the adult cuticle bears a distinct ripple pattern on each segment. Several aquatic hemimetabola share their basic body forms but have larvae that bear adaptations such as gills that are lost in the molt to terrestrial adults or unusual larval mouthparts that are radically different in the adults. The wings of hemimetabolous insects develop externally and grow gradually, visible on the outside of the body in the later larval instars. They are sometimes referred to as **exopterygotes** to reflect their external development of wings and the fact that even with incomplete metamorphosis, some body parts undergo a radical change that is uncharacteristic of hemimetabola. Some, like the lice, have secondarily lost their wings in favor of a parasitic way of life.

Holometabolous development, in contrast, is characterized by a sometimes radical change in form and ecological habits between immatures and adults (Figure 2.1). These great differences between adults and their offspring allow them to avoid intraspecific competition for food and habitat. The advantages of a holometabolous strategy are reflected in the relatively large number of insects that engage in holometabolous development, including about 770,000 species or 88% of all known insect species. Facilitating the major changes that occur during development is the intervention of an outwardly quiescent but metabolically active pupal stage, when larval structures are replaced with those of the adult. Many features, including the legs, wings, structures on the head, genitalia, and parts of the changing epidermis, arise from internal imaginal discs that differentiate and evert during the pupal stage and the metamorphosis to the adult. **Stemmata**, optical units of low resolution, are present in larvae and are replaced by the higher resolution compound eyes in adults. These stemmata may subsequently be incorporated into the margins of the adult compound eye and maintain a function in circadian rhythmicity. Ocelli are absent in larvae but may be present in adults.

The insects that engage in holometabolous development are often referred to as **endopterygotes** to reflect their internal development of wings. Some argue that the designations of exopterygote and endopterygote are better classifications than are hemimetabolous and holometabolous because not all parts of the insect necessarily conform to one developmental mode. There are some hemimetabolous insects with structures that undergo radical changes characteristic of holometabolous development such as the mouthparts of the larval dragonfly that change drastically during adult development. The terms "exopterygote" and "hemimetabola," as well as "endopterygote" and "holometabola," are used interchangeably in this text.

Endopterygote larvae generally have a relatively higher rate of growth than do exopterygotes. One reason may be that exopterygote larvae invest more material in their cuticles than do the larvae of endopterygotes. The lower growth rate of exopterygotes may reflect the increased costs involved in manufacturing the cuticle and the relatively large amounts of these materials lost in sclerotized proteins when the insects molt.

There are some predacious or parasitic insects that engage in a developmental variation of holometaboly called **heteromorphosis**, also referred to as **hypermetamorphosis**, in which successive larval instars occupy different habitats and display a marked anatomical variation. The active first instar is necessary to locate and enter the host or find food, but once the resource is located, the subsequent more inactive stages suffice. This deviation from more conventional holometaboly with two or more larval forms occurs in some Coleoptera, Hymenoptera, Neuroptera, and all Strepsiptera, with active first-instar larvae and more inactive grublike late instar larvae. In Strepsiptera, the active first-stage larva is the parasitoid's infective stage, attaching itself to a host and entering the body through

the integument. Once inside the host, it molts to a feeding stage that lacks legs but bears numerous projections that increase the surface area for the absorption of nutrients. Males emerge as free-living adults, whereas females remain as endoparasites but produce free-living first-instar larvae that seek new hosts.

ORIGINS OF HOLOMETABOLY

Ametabolous development that lacks a metamorphosis is clearly the ancestral condition but the evolutionary route that ametabolous insect ancestors took to arrive at holometaboly has been disputed. Berlese, in 1913, suggested that the holometabolous larva was a free-living embryo resulting from a premature egg hatch and that the pupal stage represented a number of nymphal stages compressed into one. The reason for the early hatch was the reduced yolk that was supposedly contained within the egg of holometabolous insects compared to the larger amount of yolk in hemimetabola. This interpretation considered the immature holometabola as being different than the immature hemimetabola and gave them different names. Berlese applied the term "nymph," a French word for "pupa," to exopterygotes to indicate that the immatures of exopterygotes were equivalent to the pupa of endopterygotes, whereas the endopterygote immature, or "larva," was a very different, free-living embryo (Figure 2.2B).

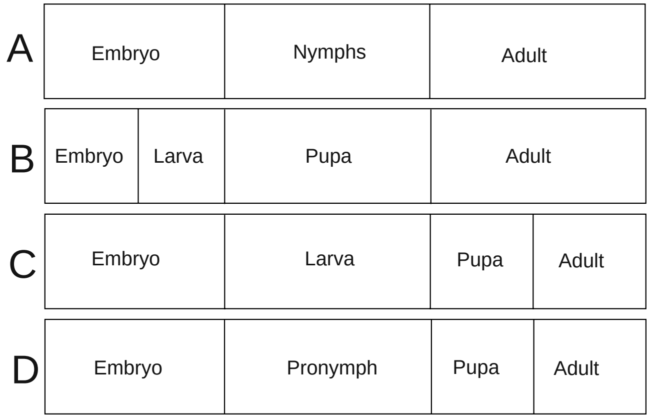

FIGURE 2.2. A. The sequence of development in exopterygotes, beginning with the embryo, the passage through several nymphal instars, and the adult stage. B. The interpretation of endopterygote development by Berlese (1913), in which the larva is considered as a free-living embryo. The pupa was equivalent to the nymphal instars of the exopterygote. C. The interpretation of endopterygote development by Hinton (1963), in which the larval instars are equivalent to the nymphal instars of exopterygotes, and the pupa was the first of two imaginal instars. D. The most recent interpretation by Truman and Riddiford (1999), in which the pronymphal stage was believed to give rise to the larval stages of the endopterygote. The nymphal stage of exopterygotes was transformed into the transitional pupal stage.

An alternative hypothesis proposed independently by Poyarkoff and Hinton, which has been more widely accepted, held that there was no difference between the immatures of exo- and endopterygotes. Because the difference that was claimed to exist between the amount of yolk in hemimetabolous and holometabolous eggs was not supported by any data, there was no developmental reason for using the different terms "larva" and "nymph" for the two immature forms. The pupa was considered to represent the first of two imaginal instars that served as a "mold" for the development of adult musculature (Figure 2.2C). In contrast, the thoracic muscles of hemimetabola were similar in the last instar larva and adult and did not require this mold. However, the mold concept was proposed without data to support it, and Hinton later modified his hypothesis to suggest the pupa was homologous to only the last instar of exopterygotes, a modification necessary to transition between the specialized early instars and the adult.

Shortly after Hinton, Wigglesworth proposed that holometabolous development was a case of sequential polymorphism in which three gene sets were activated chronologically, one each for larvae, pupae, and adults. The evolution of holometabola thus originated from gene duplication and their sequential regulation. However, this hypothesis was weakened by the demonstration that many of the proteins found in the different cuticles of the larval, pupal, and adult stages were shared, just as they were in hemimetabola, suggesting that the same gene sets were being transcribed during each holometabolous instar.

The most recent interpretation of the origin of holometabolous development by Truman and Riddiford returned to the original theory of Berlese. They pointed out a long-ignored stage in some terrestrial arthropods that exists much like a free-living embryo. This stage that they refer to as a **pronymph** in some primitive hemimetabola is proposed to be the forerunner of the holometabolous larva. The nonfeeding pronymph survives on remaining yolk and lasts for only a single instar before becoming a nymph. In extant hemimetabolous insects, the pronymphal stage occurs within the egg and the first instar nymph emerges after hatching occurs. A shift in the timing of JH secretion during embryogenesis may have been the factor promoting the evolution of the pronymph into the more stable larval stage of holometabola. In this scheme, the pronymph became involved with feeding and evolved into a larva that diverged from the adult to avoid competing with it for resources. The nymphal stage was reduced to a single nonfeeding instar that became the pupa, a transition between the larva and the adult (Figure 2.2D).

INSTARS, STADIA, AND HIDDEN PHASES

The molting cycle and metamorphosis present some interesting developmental conditions. The particular developmental stage of an insect is often arbitrarily referred to as an "instar" or "stadium," but these terms actually have more precise

meanings that are difficult to apply accurately but are important to understand. One of the first steps in molting is apolysis, in which the old cuticle separates from the epidermis and new cuticle begins to be produced. With the old cuticle no longer directly attached to the epidermis, it has effectively been discarded, although it has not been shed, and the newly formed cuticle now represents the cuticle of the next instar. For this reason, apolysis is said to mark the passage to the next instar, even though ecdysis has not yet occurred and the insect appears to still be in the skin of the earlier instar. An **instar** is therefore defined as the period between two apolyses and begins when the insect first becomes detached from its old skin (Figure 2.3).

The instar that is hidden under the old, unshed cuticle before ecdysis is referred to as the **pharate instar**. This distinction is more important for some instars than others. For example, some lepidopterans undergo diapause as pharate adults that are developmentally complete adults enclosed by the detached pupal cuticle. Although based on an external examination, it is easy to conclude that if the insect diapauses as a pupa, then the diagnosis would not be correct. The stadium that an insect is in is defined by its ecdyses; a **stadium** represents the interval between one ecdysis and the next. Therefore, at apolysis, an insect passes to another instar but does not become the next stadium until after ecdysis (Figure

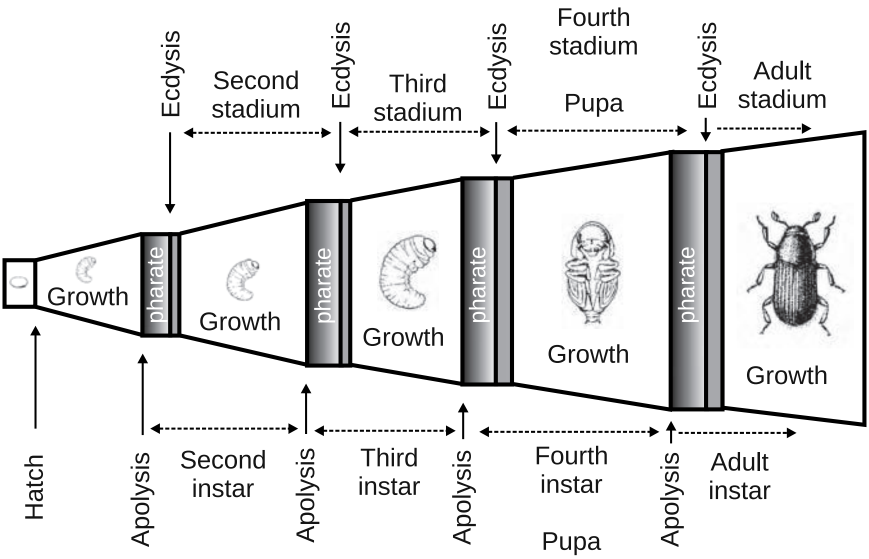

FIGURE 2.3. The molting period is punctuated by two events, apolysis and ecdysis, that define insect development. Apolysis, the separation of the epidermal cells from the cuticle, marks the beginning of the molt and the next instar. The insect is in a pharate stage until ecdysis occurs, the casting off of the old cuticle. Ecdysis marks the beginning of the next stadium. At the apolysis following the second instar, the insect enters the third instar but is still in the second stadium until after ecdysis.

2.3). These distinctions are ordinarily not discernable or even important unless one is required to assess the actual physiological state of the insect.

STRUCTURE OF THE INTEGUMENT

The outer covering of insects is referred to both as an **exoskeleton** and an **integument**. The term "exoskeleton" characterizes it based on its function as the major external support for the integrity of the insect body. Calling it an "integument" refers to it more in terms of its structure. The integument consists of the underlying basement membrane, the living epidermal cell layer, and the overlying, nonliving cuticle that is secreted by the epidermis. Given that there are more than a million identified insect species and that the specific nature of the integument of each species has exquisitely evolved along with its ecological habits, it is likely that there are also more than a million different varieties of integument. However, a general pattern of integumental structure has emerged, and it is this general pattern that will be described (Figure 2.4).

We can begin on the inside and work our way to the outside. The **basement membrane** is a continuous sheet of mucopolysaccharide, as much as 0.5 μm in thickness, and separates the epidermal cells from the body cavity. It appears to be initially secreted by plasmatocytes and may also be formed by the epidermal cells during wound repair. The basement membrane is penetrated by nerves and trachea that reach into the epidermal cell layer above.

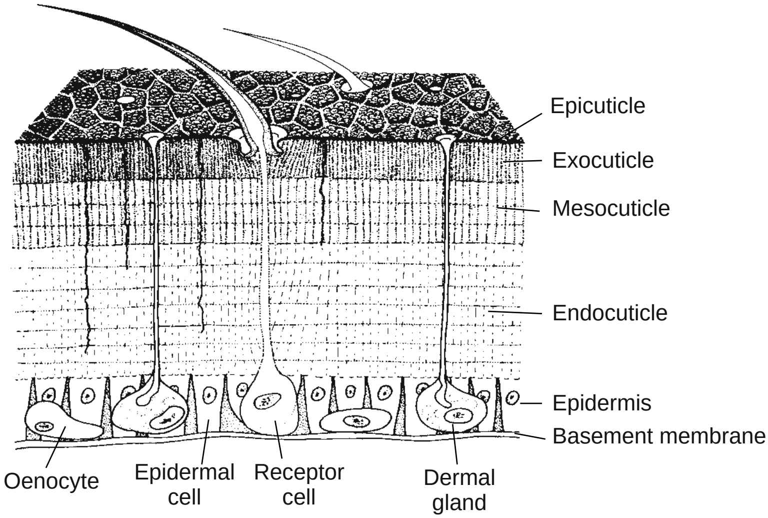

FIGURE 2.4. The generalized insect integument, consisting of the basement membrane, epidermal cells, and overlying non-living cuticle. Reprinted with permission from Hackman, R.H. (1971). *Chemical Zoology* 6: 1–62. Copyright Elsevier.

The **epidermis** that lies just above the basement membrane is the only living portion of the integument. It comprises a single layer of cells of ectodermal origin. Most of the cells are columnar, but those producing the intersegmental membrane are considerably flattened. Modifications of these cells produce such structural features as **dermal glands**, **sensory receptors** and their support cells, and **oenocytes**. Dermal glands are often multicellular, consisting of a secretory cell, a duct cell, and one or more support cells, depending on their function. They secrete the cement that covers the epicuticle and are widely distributed over the surface of the integument. They may also produce more volatile defensive secretions and pheromones that are released into the environment. Sensory receptors are specialized epidermal cells that respond to environmental stimuli and consist of structural and support cells that produce the outward form of the receptor and the internal neural dendrites that respond to specific stimuli. They are discussed in more detail in Chapter 11. Oenocytes are large polyploid cells associated with the basement membrane. Some of these oenocytes enlarge during the molting process and appear to be secretory, suggesting they are involved in the production of cuticular lipids that are deposited in the epicuticle. Other types of oenocytes may secrete ecdysteroid hormones.

Epidermal cells are bound to each other apically by **adhering zonules** and just below them, **zonular septate desmosomes** (Figure 2.5). **Gap junctions** are channels that span two cell membranes and connect the cytoplasms of neighboring cells that serve as conduits for morphogens and other small messengers. The gap junctions are encoded by a large family of tissue-specific **innexin** genes. The basement membrane is attached to the epidermal cells by **hemidesmosomes**. The *dumpy* gene encodes a large protein that anchors epidermal cells

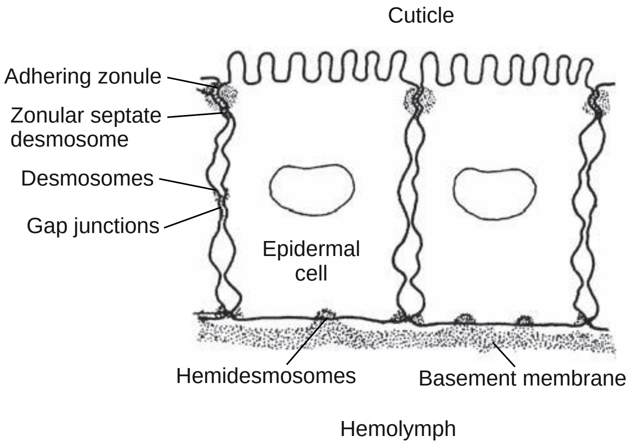

FIGURE 2.5. Coupling of epidermal cells by cell junctions. Reprinted from Locke, M. (1998). *Microscopic Anatomy of Invertebrates* 11A: 75–138. This material is used by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

and is found abundantly in areas of muscle attachment and mechanical tension. The protein mediates the anchorage of the muscles to the cuticle and organizes cuticular architecture and structural morphogenesis. Major invaginations of the epidermis during development give rise to the anterior and posterior portions of the digestive tract, the salivary glands, the trachea and tracheoles, and portions of the reproductive tracts of males and females.

While immune mechanisms are largely a function of the circulatory system (Chapter 7), the integument is the primary barrier to infection, and the epidermal cells participate in wound healing when the integument is breached. Within less than an hour after injury, epidermal cells at the site of the wound elongate with their tapering ends toward the wound. They fuse, forming a multinucleate syncytium that encircles the area. With the gap filled by a plug of coagulated hemolymph and cellular debris, other epidermal cells migrate to the puncture site and infiltrate the plug, eventually establishing an epidermal layer capable of secreting cuticle. Elevated levels of the enzymes dopa decarboxlase and a tyrosine hydroxylase are necessary for epithelial barrier formation.

The epidermal cells, along with some of the modified cells associated with them, secrete the overlying, nonliving cuticle. The cuticle is divided into three main regions: the thin outer **epicuticle**, the thick inner **procuticle** that lies just above the epidermal cells, and the **envelope**, the outermost layer of cuticle. The envelope is commonly called the cuticulin layer or outer epicuticle, and the epicuticle is deposited on its inner surface (Figure 2.6). The envelope and epicuticle thus form the upper boundary of the cuticular compartment above the epidermal cell, with the procuticle synthesized below and filling in the compartment space.

The cuticle comprises several horizontal divisions that are produced in a certain sequence during the molting process and may be developmentally altered both before and after the molt occurs. At the commencement of apolysis, the apical plasma membranes of the epidermal cells develop microvilli that are supported by a series of actin filaments. The envelope, the first component of the new cuticle to be synthesized, is laid down at the tips of the microvilli above plaques in the epidermal cell membrane into an assembly zone. After its synthesis, the envelope may be tanned and stabilized by oxidases to make it impermeable and protect the new cuticle below from the digestive enzymes that break down the old cuticle above. It may be the only cuticular layer that is present in all insects, and among the first layers of the new cuticle to be synthesized. The envelope is also the only cuticular layer found in the tracheoles. Once the envelope is laid down, the size of the insect is fixed, and in many insects that expand after ecdysis, the envelope is initially present in pleats that allow this expansion to occur.

The next layer to be formed is the epicuticle, also referred to as the inner epicuticle, which coalesces on the inner face of the envelope and is stabilized by quinones. The epicuticle is laid down from the spaces between the microvilli

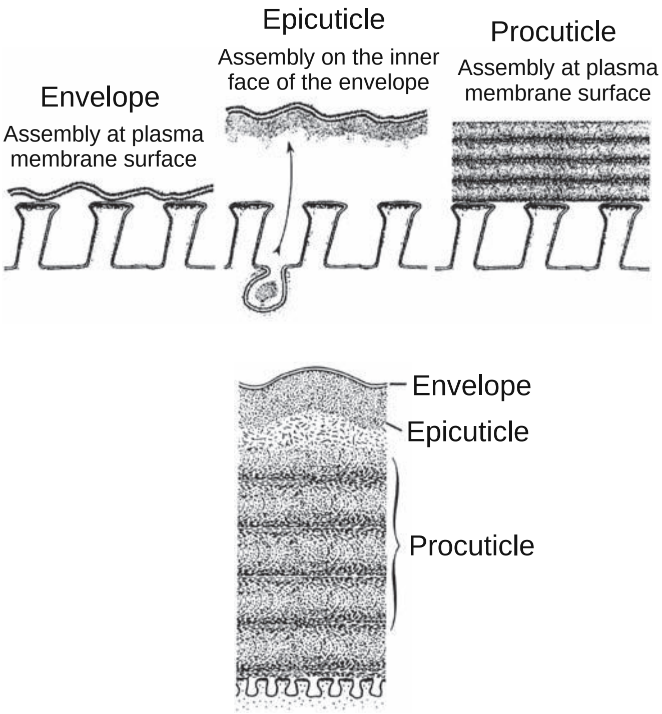

FIGURE 2.6. The assembly of the procuticle, epicuticle, and envelope from the membrane of epidermal cells. Reprinted with permission from Locke, M. (2001). *Journal of Insect Physiology* 47: 495–507. Copyright Elsevier.

and ranges from 0.5 to 2 μm in thickness. Its exact composition has yet to be determined, but it contains both polyphenols and the enzyme polyphenol oxidase that are involved in tanning the cuticle. It may also be involved in wound repair, tanning scratches that may occur.

The procuticle is the region of the cuticle that contains chitin; no chitin has yet to be found within the epicuticular layer. The procuticle is secreted at the tips of epidermal cell microvilli as stacks of laminae and consists largely of chitin and protein that is initially undifferentiated and not chemically cross-linked. Each lamina consists of a layer of parallel chitin chains, with the orientation of the chains within each lamina changing to yield an overall helicoidal pattern (Figure 2.7). As the molting cycle proceeds, at least two and sometimes three regions of the procuticle can be identified. The first portion of the procuticle to be synthesized becomes the outer **exocuticle**, a region in which the proteins eventually become heavily cross-linked and insoluble. This insolubility prevents the exocuticle from being broken down during the molting cycle so the portion that is shed during ecdysis consists mostly of exocuticle. The **endocuticle** is the portion of the procuticle that lies just above the epidermal cells, ranging from

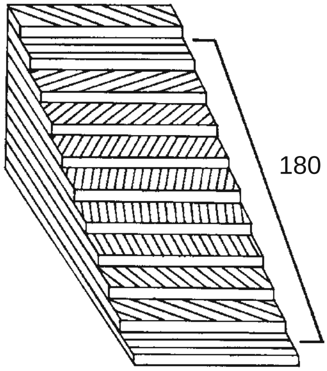

FIGURE 2.7. The helicoidal arrangement of the chitin layers as they are rotated by a constant angle during their synthesis and deposition. The bar shows the rotation of layers through 180°. From Neville (1984) Reprinted with permission.

10 to 200 μm in thickness, consisting of several lamellar layers. Its synthesis continues after the old cuticle is shed, often in daily layers that can be used to age-grade adult insects. The endocuticle, without much developed exocuticle, comprises most of the cuticle that is present in soft-bodied insects and in areas of flexibility such as the regions between body segments and joints. In contrast to the exocuticle, in the endocuticle the cross-linking of proteins is reduced, allowing it to be completely broken down by enzymes and resorbed during the molting process. These differences in chemical cross-linking of cuticular proteins give the exocuticle and endocuticle their particular properties. **Mesocuticle** has been identified in several insects and is interpreted as a transitional layer in which the proteins are untanned like the endocuticle but impregnated with lipids and proteins like the exocuticle.

Other products of the epidermal cell and dermal glands are secreted to the outside of the envelope. The **cement layer** consists mostly of lipoprotein that is secreted by different groups of dermal glands whose contents are discharged and mix on the surface, but the composition and thickness of the layer varies from species to species. In spite of its alleged importance, it is completely absent in the cuticle of honey bees. It may serve as a varnish to coat and protect the wax layer just beneath it. Underneath the cement layer is the **wax layer**, produced by the epidermal cells and transported to the surface of the cuticle through the pore canals that permeate the procuticle. Cuticular waxes are mixtures of hydrocarbons with 25 to 31 carbon atoms, alcohols of 24 to 34 carbon atoms, and esters of fatty acids. One major role of the wax layer is in waterproofing. It is generally absent in aquatic insects and is also lacking in other arthropods such as centipedes and millipedes that are confined to humid environments and inactive during the heat of the day. When insects are exposed to high

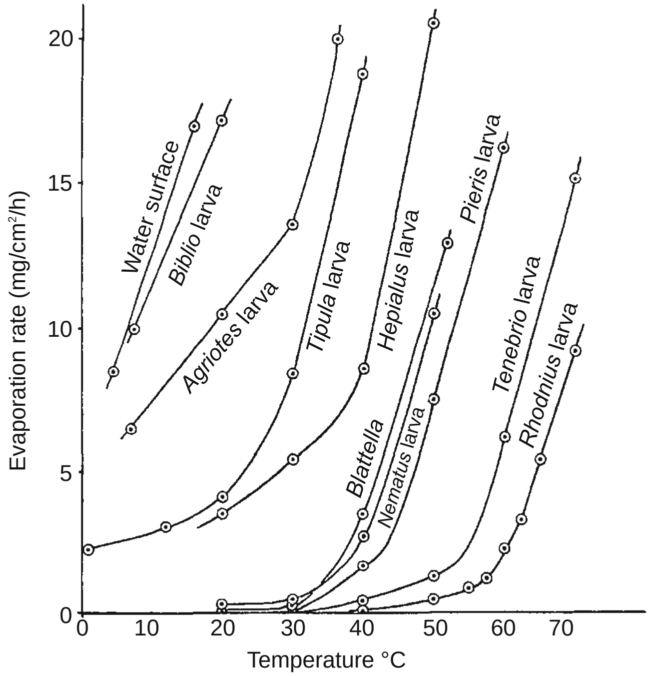

FIGURE 2.8. Evaporation rate of water through the cuticles of several insect species. The rates are a function of temperature and properties of the waxes present. Adapted from Wigglesworth (1945).

temperatures that are above a transition point that represents the phase change or melting of the cuticular waxes, water loss increases significantly as a result of the disorientation of the wax layer. The transition point is a function of the composition of the cuticular waxes that are present and differs for different species (Figure 2.8).

MODIFIED FEATURES OF THE INTEGUMENT

The vertical organization of the integument that has been described does not necessarily apply to all areas of the insect. Depending on the functions of specific regions, there may be some layers that are either absent or more prominent. **Arthrodial membrane**, also called the **intersegmental membrane**, is the flexible membrane between body segments where the exocuticle is absent (Figure 2.9). The untanned endocuticle that remains contains special acidic proteins and the flexible protein resilin that provide the flexibility in the region.

Ecdysial lines are also areas of reduced exocuticle, but they differ from arthrodial membrane in that they are programmed areas of weakness that serve

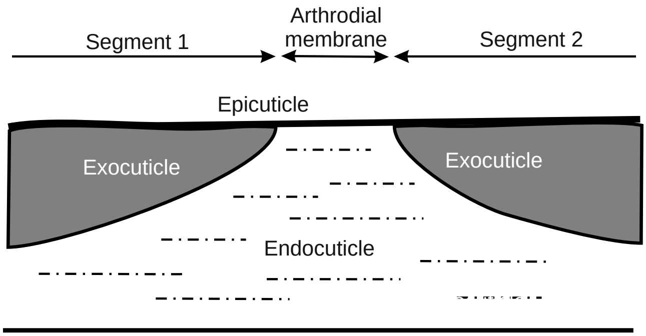

FIGURE 2.9. A cross-section of the integument between two segments, showing the absence of exocuticle that results in the flexible arthrodial membrane.

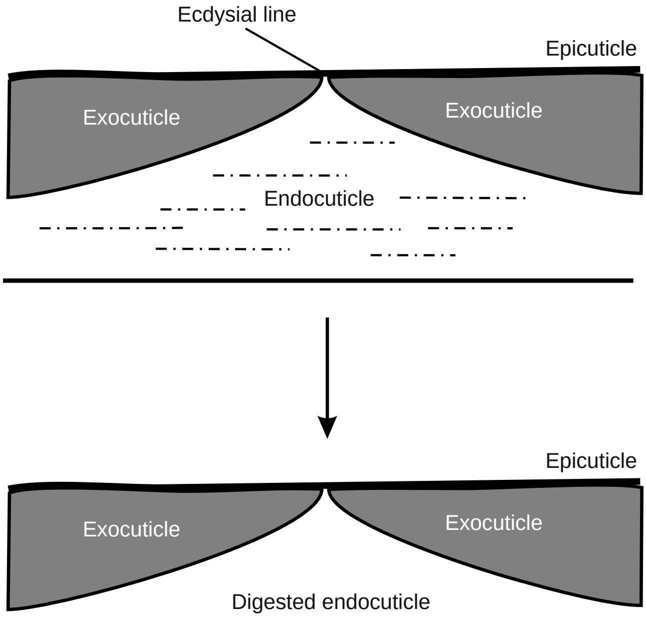

FIGURE 2.10. A cross-section of the integument in an area that is programmed to split during ecdysis. The absence of exocuticle and the digestion of the endocuticle that remains allow the insect to easily break the epicuticle that remains for emergence.

as emergence points during ecdysis. When the endocuticle is digested during the molting cycle, only a small layer of epicuticle remains that can be disrupted when the new integument is expanded (Figure 2.10). Soft-bodied insects such as caterpillars have cuticles with reduced exocuticle that provides the flexibility and the potential for growth that they require during an instar. Specific areas of programmed weakness can be disrupted when some insects are threatened by

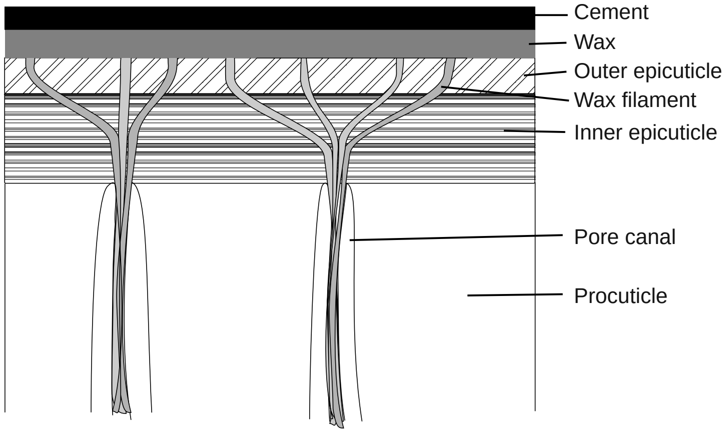

FIGURE 2.11. The pore canals that extend from the epidermis to the surface of the cuticle.

predators. In **reflexive bleeding**, insects increase their internal pressure to rupture the integument at localized points and release a droplet of distasteful hemolymph that discourages the predators. The integumental rupture is able to suck back the hemolymph and heal within minutes. With **easy bleeding** that occurs in some sawfly larvae, the integument of the entire body can potentially rupture from an external, rather than an internal, force. Easy bleeders have spider-like microstructures on the surface of the cuticle that may facilitate the disruption of the integument and make the surface of the cuticle hydrophobic, so the exuded hemolymph remains as a droplet and does not spread over the surface.

Pore canals extend from the epidermis through the cuticle to its surface (Figure 2.11). They are cytoplasmic extensions of the epidermal cells, rising in a helical pattern as they travel through the procuticle. Although they are not covered by the epidermal cell membrane, they do contain filaments from the cell. In the cockroach integument, approximately 200 pore canals arise from each epidermal cell, equivalent to 1.2 million/mm^2. The pore canals transport lipids produced by the epidermal cells to the surface of the newly formed envelope. Pore canals are absent in transparent cuticles, such as those that cover the compound eyes.

CHEMISTRY OF THE CUTICLE

The insect cuticle is composed largely of proteins, lipids, and chitin. The proteins and chitin interact to provide the mechanical function of the cuticle, conferring

the strength and hardness that is necessary for it to serve as an exoskeleton. Lipids provide protective and communicative roles, located mostly on the outermost layers of the cuticle, and are secreted once the rest of the cuticle has been produced.

Proteins

Proteins can often constitute more than half the dry weight of the insect cuticle. They are primarily located within the procuticle, but the epicuticle also contains several minor proteins that are relatively difficult to extract. The stage-specific properties of insect cuticles are largely derived from the distinct stage-specific proteins that are synthesized and the manner in which they interact with the lipids that are present. Cuticular proteins are synthesized mainly by epidermal cells according to a temporal pattern during the molting process, and the sequence of their synthesis may even extend throughout the instar to change the nature of the cuticle during the instar. Unlike the chitin in the laminae of the procuticle, the location of the proteins bears little relationship to their time of secretion.

Early studies of cuticular structure referred to a single protein called **arthropodin** that comprised the bulk of the cuticular proteins. However, the term "arthropodin" was no longer used once it became clear that a diversity of cuticular proteins was actually present. Many of the proteins found within insect cuticles differ from each other by only a few amino acids and are considered as variants of the same protein, sharing a common evolutionary origin and placed in the same family. A large number of the cuticular proteins identified to date can be placed into one of three protein families. Proteins from flexible areas of insect cuticles that do not become sclerotized contain a conserved amino acid sequence known as the Rebers-Riddiford consensus sequence (RR-1), named after the study that first identified them in *Manduca* and *Drosophila*. Those proteins from hard sclerotized cuticles lack the RR-1 sequence but may instead contain a variant RR-2 sequence. Both the RR-1 and RR-2 regions of these proteins may be involved in the binding of the proteins to chitin, because they are likely to undergo a folding that places the binding regions on the same side of the molecule. Five proteins derived from the postecdysial cuticles of a variety of arthropods are additionally categorized as RR-3 variants. The cuticular proteins that contain the RR-1 or RR-2 regions are bound to the chitin chains, whereas those that lack the regions tend to be located in the spaces between the chitin, forming a matrix of proteins and water molecules (Figure 2.12). More than 400 different cuticular protein sequences have been identified from six insect orders.

Other proteins contain repeated hydrophobic amino acid sequences (Ala-Ala-Pro-Ala/Val) that undergo frequent structural turns resulting in a spiral molecule

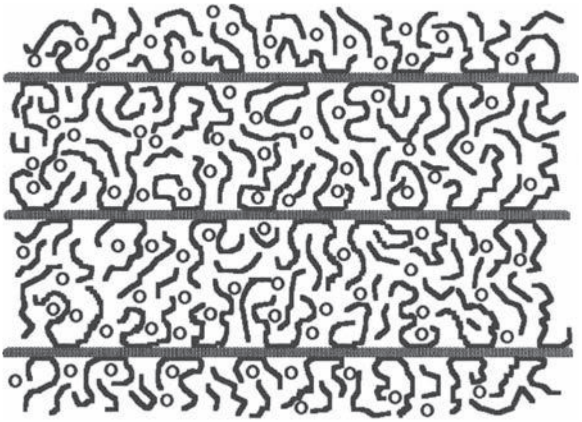

FIGURE 2.12. The matrix of protein (short chains) and chitin filaments (gray bands) that associate in the procuticle. Water molecules are shown as small circles. (From Andersen, 2000).

that is easily deformable. Areas of the cuticle that have an unusual elasticity contain the protein **resilin** (named from the Latin *resilire*, to jump back), which possesses a rubber-like property imparted by the coiling of its protein chains that are linked by di- and trityrosine residues. It also contains a high percentage of glycine and proline residues and an amino acid sequence that minimizes cross-linking with any other proteins that could reduce its elasticity. Resilin has been called an almost perfect rubber that permits the storage of energy in leg joints, in the thoracic body wall, and in wing hinges for locomotion. Insect flight is possible because energy that is stored in resilin during the upstroke is released to supplement the energy from muscles during the power-producing downstroke. The pharyngeal pumps in the digestive tracts of sucking insects are supplied with resilin, allowing them to expand with muscle contraction and contract from the energy stored in the elasticity.

The proteins can also be classified into families based on their production and transport (Figure 2.13). The more traditional class C cuticular proteins are synthesized by epidermal cells and secreted into the cuticle above them. Class H proteins are secreted only into the hemolymph, in the other direction. Class BD (bidirectional) proteins are synthesized by epidermal cells and are secreted both into the cuticle and into the hemolymph. The blue pigment insecticyanin is an example of a BD protein that is located in the epidermis, cuticle, and hemolymph of *Manduca*. Class T proteins are transported across the epidermal cells from the hemolymph into the cuticle and may be synthesized by hemocytes or at other nonepidermal sites. This cuticular protein classification is based entirely on their routing and not their function; proteins within each of these classes may have functions unrelated to others within their group. The classification recognizes that the epidermal cells are capable of both the synthesis as well

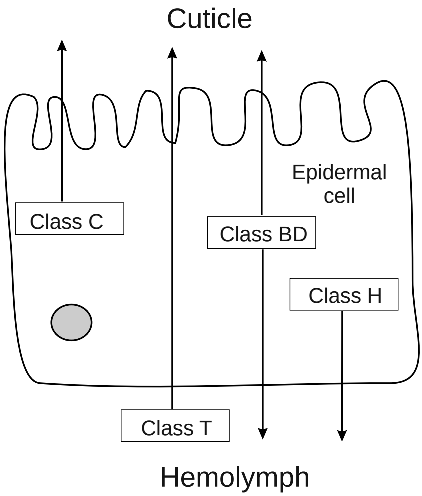

FIGURE 2.13. Families of cuticular proteins based on their transport. The usual Class C proteins are synthesized by the epidermal cells and deposited into the cuticle. Class T proteins are taken up from the hemolymph by the epidermal cells and deposited in the cuticle. Class H proteins are synthesized by the cells and secreted only into the hemolymph, while Class BD proteins are transported in both directions. (After Sass et al., 1993).

as the transport of proteins that are destined to end up in the cuticle and hemolymph.

Although a temporal pattern of cuticular protein synthesis exists within each instar, with specific proteins synthesized and deposited in the cuticle at specific times during and after the molting cycle, the location of proteins within the cuticle does not necessarily correlate with the time of secretion. Proteins that are produced may diffuse through the cuticle as they are deposited. Many of the proteins are common within the cuticles of several instars. For example, of the 152 electrophoretic bands representing cuticular proteins of *Hyalophora cecropia*, 7% were only found in larvae, 15% were only found in pupae, and 9% were characteristic of adults. The different types of proteins present and their degree of sclerotization is related more to the type of cuticle produced in a specific area than it is to any species or instar relationships. Heavily sclerotized cuticles contain a large proportion of hydrophobic, positively charged proteins. Flexible cuticles tend to be associated with the presence of more acidic proteins that also have a higher capacity to bind water.

The character of some proteins can be altered even after the cuticle has been synthesized. Although the cuticle of the blood-sucking bug *Rhodnius prolixus* is normally stiff, when a blood meal begins to be ingested, endocrine events lower the pH of portions of the cuticle to below 6. With a more acidic pH, the conformation of the proteins is altered and they become more plastic, allowing the

insect to expand its abdomen and accommodate the large blood meal. Cuticles can be hard even in the absence of chitin. Manipulating cuticular water content can affect its stiffness; the removal of water from the cuticle results in the proteins forming more β-sheets, which are cross-linked by interchain hydrogen bonds. Covalent bonds between proteins produce the hard, stabilized cuticles that are found in insect mandibles with additional hardness from the minerals manganese and zinc. In the oothecae of cockroaches and mantids, protein cross-linking alone accounts for the hardness and tanning of the structural proteins. The asymmetrical female accessory glands produce products that sclerotize the structural proteins when they mix with enzymes in the genital vestibule (Figure 2.14). The process of cuticular sclerotization is discussed in more detail in a later section.

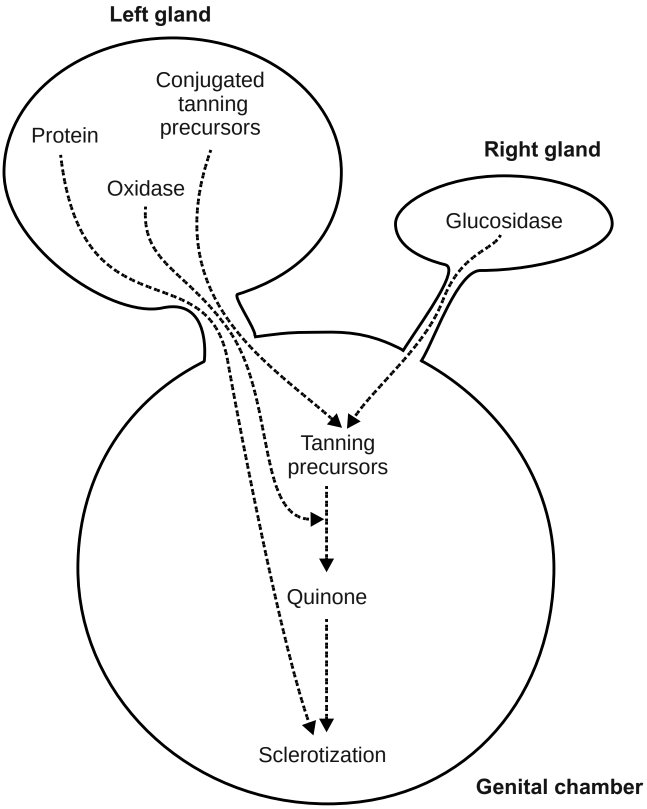

FIGURE 2.14. The mechanism of ootheca production in the cockroach. The left gland secretes oothecal proteins, tanning precursors that are conjugated and inactive, and an oxidase. The right gland secretes a glucosidase. When the contents are mixed in the genital chamber, the glucosidase removes the conjugate and makes the tanning precursors available to the action of the oxidase. The oothecal proteins are sclerotized by the quinones that result.

Insect coloration may be the result of the absorption of certain wavelengths of light by protein pigments. Some of the pigments may be synthesized by the insect and deposited in the cuticle. These include the ommochromes, pteridines, melanins, porphyrins, and bile pigments. Other pigments cannot be synthesized and must be derived from plant products that are ingested. These include the carotenoid and flavenoid pigments. The common black and brown coloration in many insects is the result of either quinone sclerotization of cuticular proteins or the deposition of melanin pigments in the epidermal cells. Melanism is an important mechanism that allows insects living at higher altitudes and latitudes to absorb more solar radiation so they may heat up more quickly to maintain activity levels.

The strikingly bright cuticular coloration of some insects is often the result of the reflecting structural properties of the cuticles and not to any protein pigments. This physical color is based on a structural order in the cuticle that manipulates reflected light by interference so the wavelengths are reinforced or diminished. Many neotropical butterflies in the genus *Morpho* have blue and green metallic coloration that results from finely lamellated scales reflecting light repeatedly at successive layers and causing this interference (Figure 2.15). Their intensely bright coloration allows them to be visible from over a quarter mile away, facilitating conspecific signaling.

Chitin

Produced by epidermal and midgut endodermal cells, chitin is the other major component of the procuticle, varying from 10% to 45% of the total dry weight of the cuticle depending on species and stage. It is also a component of the peritrophic matrix that lines the midgut. In *Manduca sexta*, larval, pupal, and adult cuticles contain 14%, 25%, and 7% chitin, respectively. In addition to its presence in the cuticle of arthropods, it is involved in the formation of the skeletons of annelids and mollusks and is also produced by some protozoans and fungi. As the most common amino polysaccharide found in nature, it rivals cellulose in its annual production.

Chitin is an acetylated polysaccharide similar to cellulose. It is a polymer of N-acetyl-D-glucosamine with a few additional residues of unacetylated glucosamine and is connected by unbranched β-1,4 linkages, which make the chitin chains ribbon-like (Figure 2.16). Adjacent chains of chitin are cross-linked by hydrogen bonds to form chitin microfibrils about 3 nm in thickness, with an estimated 19 chitin chains of 0.3 μm length in each microfibril. The microfibrils are known to exist in α, β, and γ crystallographic orientations that differ in the alignment of the chitin chains, but only the antiparallel **α-chitin** form is overwhelmingly present in insects (Figure 2.17). The microfibrils are laid down in a parallel orientation within a single layer, but the orientation in successive layers

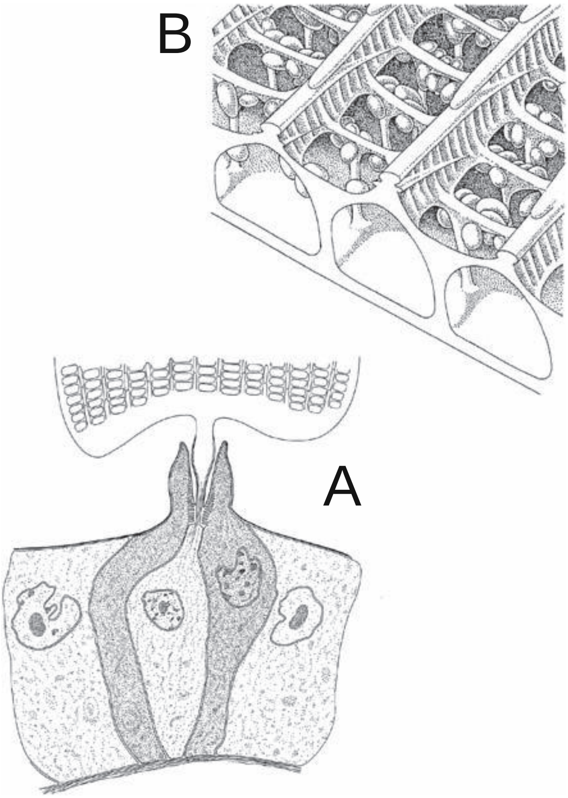

FIGURE 2.15. (A) An epidermal cell on a lepidopteran wing forming a scale. The ribs and lamellae on the scale (B) diffract the light and produce structural colors. Reprinted with permission from Ghiradella (1991).

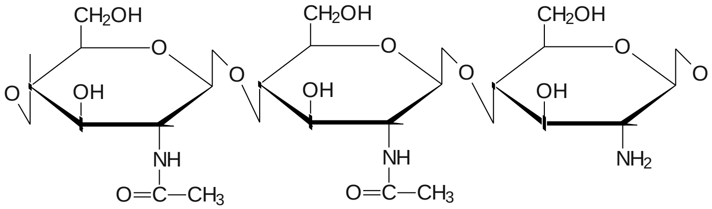

FIGURE 2.16. A portion of the chitin chain, showing two residues of N-acetyl-D-glucosamine and one of glucosamine.

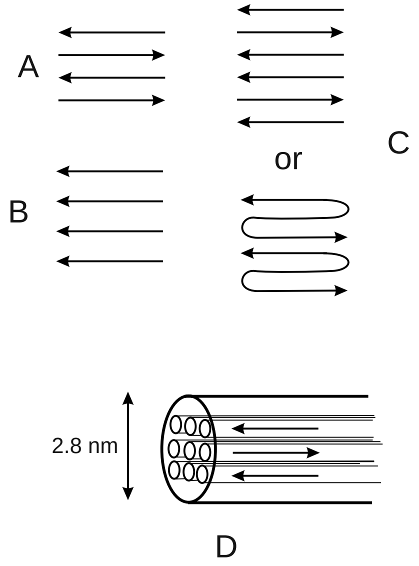

FIGURE 2.17. The orientation of the chitin chains in the cuticle. A. The orientation of α chitin, the most common form in insects. B. The orientation of β chitin. C. Two possible orientations of γ chitin. (After Rudall, 1963). D. The location of the chains of α chitin in a single chitin microfibril (After Reynolds, 1987).

is rotated by a constant angle to produce a plywood-like helicoidal arrangement that contributes to its overall strength (Figure 2.7). The chitin microfibrils are covalently linked to the surrounding proteins, with the relative amounts of protein and chitin varying between insect species and from one area of the cuticle to another within the same insect. Chitin is always associated with cuticular proteins.

Chitin has an unusual chemical stability, being insoluble in water, dilute acids, concentrated alkali, alcohol, and organic solvents. In concentrated alkali at high temperatures, the acetyl groups are detached and chitosan is formed. The steps in the synthesis of chitin have become important and better understood as a consequence of the discovery of a class of compounds that blocks chitin formation and causes insects to die because they fail to produce cuticles of suboptimal strength. Chitin synthesis begins with glycogen released from the fat body. The enzyme glycogen phosphorylase converts this to glucose-1-phosphate with the ultimate release of trehalose into the hemolymph. The trehalose is acted on by the enzyme trehalase to produce glucose that is phosphorylated, aminated, and acetylated, forming a monomer that is activated by uridine diphosphate and added to the end of an existing chitin chain. It is degraded during the molting cycle by chitinases that are present in the molting fluid and can be recycled for its resynthesis (Figure 2.18). The digestion products within the molting fluid are

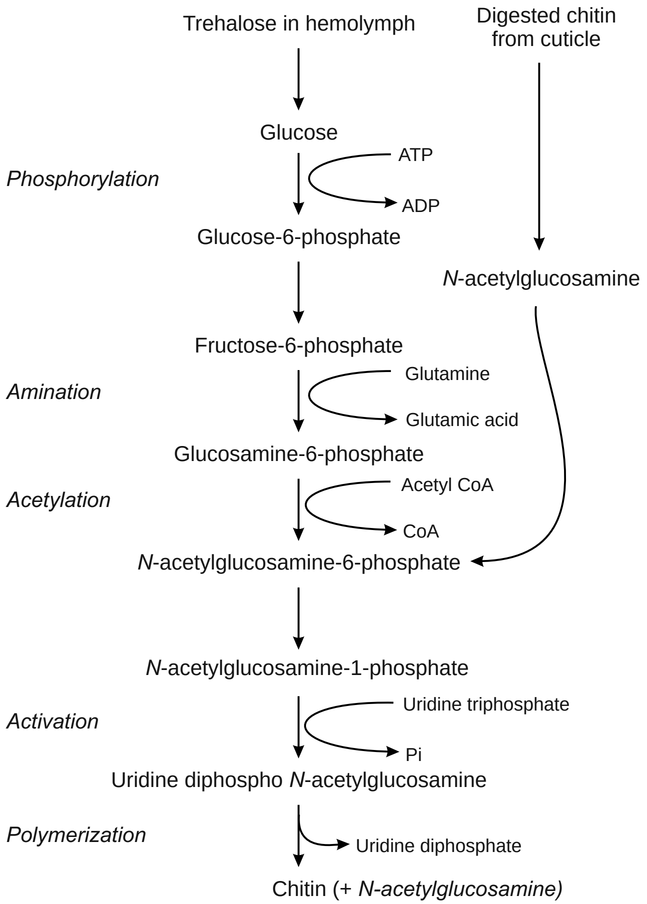

FIGURE 2.18. The steps in chitin biosynthesis.

mainly transported to the midgut by way of the mouth and anus, but some direct resorption by epidermal cells may also occur.

Interference with chitin synthesis and degradation has been considered as a tactic for insect control. Inhibitors of chitin synthesis include the **peptidyl nucleosidases**, the **acyl ureas**, and several hormone agonists. The peptidyl nucleosidases are substrate analogs that inhibit the enzyme chitin synthase. The acyl ureas, diflubenzuron and teflubenzuron, inhibit chitin synthesis by affecting epidermal cell transport mechanisms. The ecdysteroid agonist tebufenozide, and the JH agonists fenoxycarb, pyriproxyfen, and methoprene, interfere with chitin synthesis by interacting with the hormone receptors (Figure 1.34). Chitinase inhibitors may mimic the carbohydrate substrate. Because the malaria parasite

also uses a chitinase to penetrate the peritrophic matrix of the mosquito midgut, chitinase inhibitors also can block malaria transmission. **Calcofluor**, a chemical with chitin binding properties, can inhibit the formation of the peritrophic matrix by disrupting the chitin-protein interactions.

Lipids

The bulk of the lipids present in the cuticle are localized in the wax layer above the envelope where they prevent desiccation and provide chemical cues for species recognition. As the primary barrier to the penetration of environmental chemicals, the nature of cuticular lipids must be understood in order to design effective contact insecticides that are able to cross this barrier. They exist in a solid state within the cuticle, but at elevated temperatures they may undergo a phase change. Although water loss is modest at low temperatures, once the lipids reach this transition temperature the water loss increases substantially (Figure 2.8). A number of hydrocarbons act as sex pheromones and cues for caste recognition in social insects. Eggs laid by queen ants can be recognized as such by the workers by virtue of their surface hydrocarbons. Some insects secrete prodigious quantities of wax in addition to the lipid deposited in the cuticle. Honey bees produce wax from abdominal epidermal glands to build the honeycomb of the hive, and scale insects use the wax they produce, deposited as "blooms," for protection from predators and from desiccation.

The lipids in the cuticle are synthesized largely by the oenocytes and the cells of the fat body and then are taken up by the epidermal cells for their distribution through the pore canals to the surface of the integument. Because the lipids are insoluble in the aqueous hemolymph, they must be transported by special protein molecules called **lipophorins** that act as reusable lipid shuttles (see Chapter 6). A number of different lipids are found in the cuticle, commonly mixtures of *n*-alkanes, *n*-alkenes, di- and trimethylalkanes, and monomethylalkanes.

Other Components of the Cuticle

Phenols, largely derived from tyrosine metabolism, have been identified from arthropod cuticles. These are generally involved in the tanning reactions within the procuticle that stabilize the protein matrix. A number of enzymes related to cuticular tanning are also present, including various phenoloxidases. There is usually nothing more than a trace amount of inorganic components, but some insects, including the pupae of the dipteran *Musca autumnalis*, deposit large amounts of calcium. Calcium carbonate also accumulates in the Malpighian tubules of the larvae and is incorporated into the puparium as a means of

cuticular hardening. Some hymenopterous parasitoids find their sequestered hosts by drilling through hardened substrates such as wood using their long ovipositors. The deposition of manganese and zinc at the tips of the ovipositors allows them to withstand the wear and tear as well as the enormous forces placed on them. High levels of Mn and Zn are also present at the cutting edges of the mandibles of herbivorous insects such as many locusts and caterpillars to increase their hardness and reduce the abrasive wear.

Sclerotization

Cuticular **sclerotization**, also known as tanning, stabilizes the protein matrix of the cuticle to make it stiffer and harder, more insoluble, and more resistant to degradation. The process gives the integument greater strength for muscle attachment and locomotion and provides stability against hydrolytic enzymes produced by potential pathogens such as fungi. The process of sclerotization cross-links the functional groups of cuticular proteins when they react with quinones.

During sclerotization, the epidermal cells secrete several agents into the cuticle where they are transformed into more reactive compounds that are able to link the proteins. The amino acid tyrosine provides one of the precursors for sclerotization. Insects cannot synthesize the phenyl ring of tyrosine, so it must be acquired in the diet and is sequestered in the hemolymph as conjugates of glucose or phosphate. Tyrosine has a low solubility, and this conjugation may be necessary to increase its solubility in the hemolymph and protect it from being used in other metabolic reactions. The precursors for sclerotization are derived from tyrosine in three enzymatic steps (Figure 2.19). First, the tyrosine is hydroxylated to **3,4 dihydroxyphenylalanine** (DOPA). The DOPA is then decarboxylated to **dopamine**, followed by the acylation of the dopamine amino group with either acetate or β-alanine to form the catecholamines ***N*-acetyl dopamine** (NADA) or ***N*-β-alanyldopamine** (NBAD). The character of the tanned cuticle is determined by which of these tanning precursors is selected. Areas containing high concentrations of NADA tend to be less dark than those containing NBAD. The darkening of the cuticle otherwise occurs when some of the dopamine is channeled into the pathway for melanin production. The conversion of NBAD back to dopamine by NBAD hydrolase provides more dopamine for this pathway.

The catecholamines are oxidized by two classes of phenoloxidases, the tyrosinases and laccases, once they are released into the cuticle to form reactive quinones. In quinone sclerotization, the *o*-diphenols are oxidized to *o*-quinones. In β-sclerotization, another pathway that produces cross-linking agents, the β-carbon of the aliphatic side chain, is activated (Figure 2.20). In both cases, the -NH2 and -SH groups on the proteins bond to the tanning agents. In addition

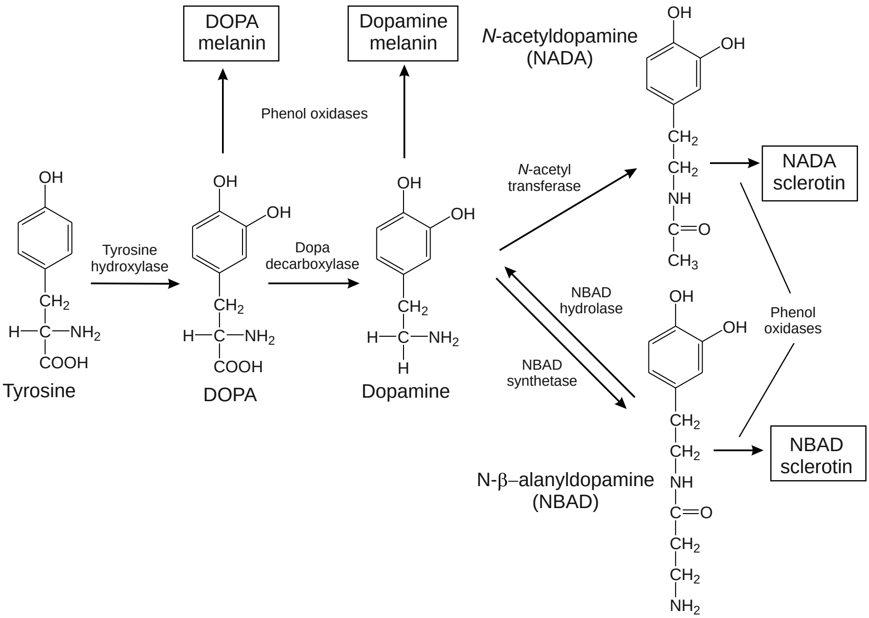

FIGURE 2.19. The steps in the synthesis of cuticular tanning precursors.

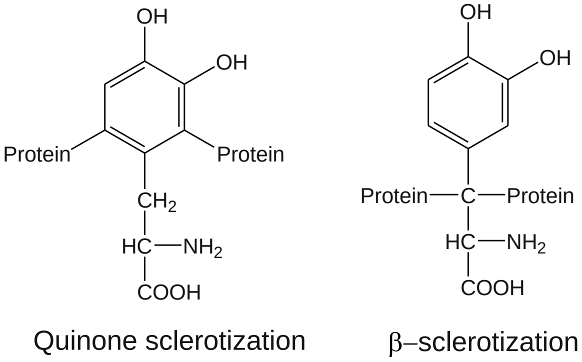

FIGURE 2.20. Differences between quinone sclerotization and β-sclerotization in where the cross-linked proteins are attached.

to the association of NADA or NBAD with the type of cuticle produced, the covalent bonds that form between either the aromatic or side chain carbons also determine the type of sclerotized protein that results. The regulation of gene expression by the individual epidermal cells produces the enzymes, proteins, and

tanning precursors that are present in localized areas of the cuticle and determine where and when the process of sclerotization occurs.

Sclerotization is regulated by at least two hormones. Ecdysteroids induce the epidermal cells to synthesize the enzyme **dopa decarboxylase** that is involved in the pathway toward the synthesis of NADA. The declining ecdysteroid titers that follow ecdysis then induce the release of the neurohormone **bursicon**, which increases the permeability of epidermal cells to tyrosine and catecholamines, allowing for the production of the tanning agents. Bursicon is synthesized in neurosecretory cells in the brain and ganglia of the ventral nerve cord as a heterodimeric protein comprised of two cystine knot polypeptide subunits. The genes that encode the subunits, *pburs* and *burs*, are homologous to the bone morphogenetic protein anatagonist gene family that is involved in embryonic development in vertebrates.

THE MOLTING PROCESS

Although many holometabolous larvae can grow between molts, substantial growth in insects occurs only during and immediately after the molting process. Unsclerotized cuticle can accommodate some expansion for growth during an instar, but its stretching is limited by the extensibility of the folds in the epicuticle. Sclerotized cuticle is largely incapable of expansion beyond the limits set by the envelope. The molting process involves an elaborate sequence of events that produces a new cuticle capable of significant expansion before the old one is discarded. The process begins with the separation of the epidermal cells from the old cuticle, known as **apolysis**, and ends with the casting off of the old cuticle, known as **ecdysis**. It is the period between these two events when the components of the new cuticle are synthesized (Figure 2.21).

During the intermolt period, the epidermal cells are attached to the cuticle by plaques that anchor the base of the cuticle to the microvilli. At the beginning of the molting cycle, the epidermal cells undergo mitotic growth and increase their density and shape when they are stimulated by hormones released by the endocrine system. This causes apolysis to occur, the separation of the cuticle from the epidermis, and creates an area between the cuticle and epidermis, the **exuvial space**. The exuvial space fills with a **molting gel** that contains inactive enzymes including a chitinase and protease that will be capable of digesting the old cuticle above once they are activated. Soon afterward, the epidermal cells secrete a new envelope whose lipoproteins become tanned and impervious to these degradative enzymes when they later become activated by factors produced by the epidermal cells. The active enzymes, now called the **molting fluid**, are prevented from attacking the epidermal cells by the barrier provided by the outer epicuticle layer. The molting fluid is now free to begin the digestion of the old

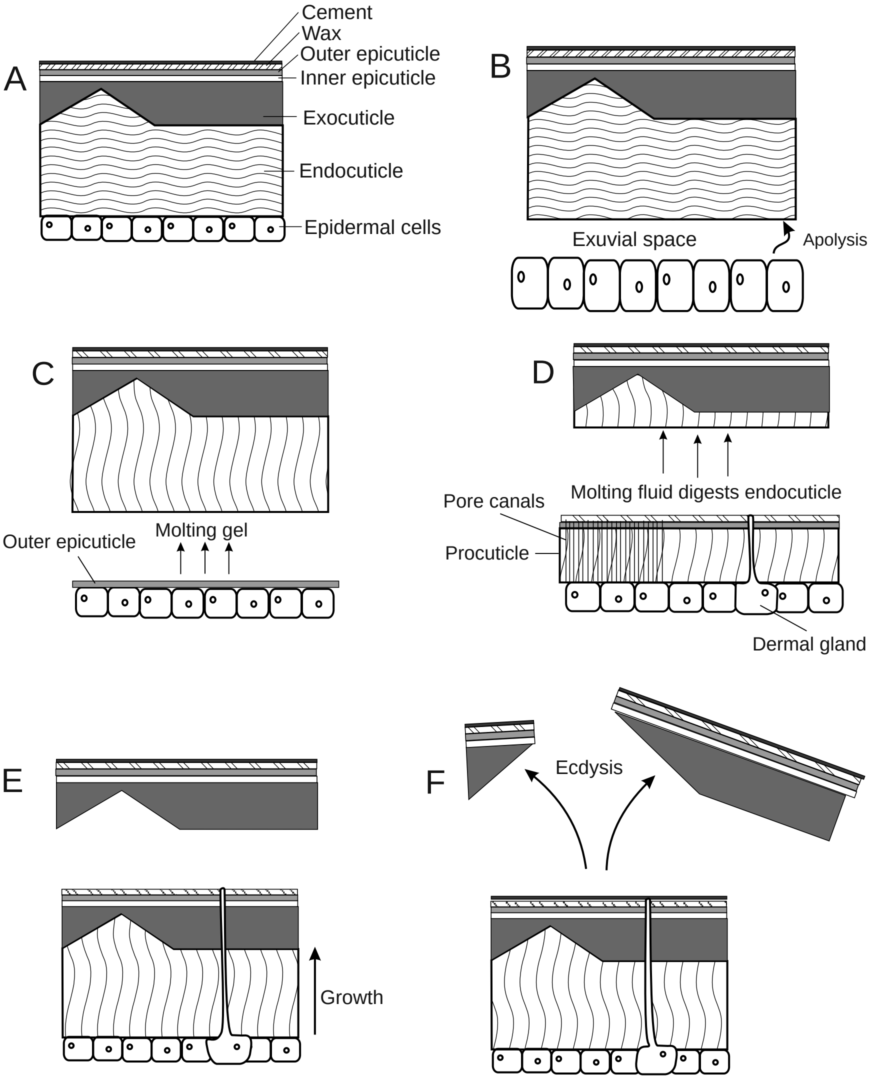

FIGURE 2.21. Steps in the molting process. A. The integument before the molt. B. Apolysis, separating the cuticle from the epidermis and creating an exuvial space. C. Secretion of the molting inactive molting gel into the exuvial space. D. The digestion of the old endocuticle and the secretion of new procuticle. E. Continued growth of the procuticle and epicuticle. F. Ecdysis, the shedding of the old cuticle.

unsclerotized endocuticle, but not the sclerotized exocuticle. About 90% of the materials in the old endocuticle may be resorbed and reused.

As the old cuticle is digested, the epidermal cells begin to secrete the new procuticle using the recycled raw materials. The synthesis of the new epicuticle continues with the formation of the inner epicuticle. With only the exocuticle remaining after digestion, the ecdysial lines become important points of weakness in the old cuticle. Just before ecdysis, the pore canals release the components of the wax layer on the surface of the outer epicuticle and assure the waterproofing of the new cuticle before the old cuticle is discarded. The molting fluid is then completely resorbed. A complex sequence of stereotyped behaviors follows, initiated by ecdysis-triggering hormone and eclosion hormone, which ultimately cause the shedding of the old cuticle after the insect swallows air or water that expands its body and splits the old exocuticle along the ecdysial lines.

Following ecdysis, the new cuticle is soft and unsclerotized. It expands within the limits already set down by the outer epicuticle to express the increase in size that was the reason for the molt. The dermal glands release their products to the surface and create the cement layer that covers the wax layer. The tanning precursors are released into the new cuticle by the epidermal cells, and the differentiation of the procuticle into the endocuticle and exocuticle is initiated. Growth of the endocuticle continues after ecdysis in many insects and does not stop until apolysis and the next molting cycle.

The epidermis is not the only portion of the insect that molts. All cells that are epidermal derivatives go through the same steps of apolysis through ecdysis. This includes the cells that make up the foregut, hindgut, and tracheal system.

Eclosion Behavior and Its Endocrine Regulation

The stereotyped behaviors that allow the insect to shed its old cuticle occur only at specific times in the life cycle. In the well-studied *Manduca sexta*, the behaviors associated with ecdysis have been divided into two phases: preecdysis behavior and ecdysis behavior, both of which appear to be programmed into the central nervous system. During preecdysis behavior, the connections to the old cuticle are loosened through rotational movements of the abdomen. During ecdysis behavior, the loosened cuticle is shed by means of peristaltic contractions that move anteriorly along the length of the larva.

A cascade of several neurohormones is responsible for eliciting eclosion behavior. At the appropriate time in the insect's life cycle, the brain releases **corazonin**, causing the release of **ecdysis-triggering hormone** (ETH) from Inka cells that are located on the trachea. Both the release of ETH and the sensitivity of the nervous system to the hormone depend on the declining titers of ecdysone after the molting peak. ETH acts on the cells of the ventral nerve cord, causing the initial expression of preecdysis behavior and the release of

another hormone, **eclosion hormone** (EH), from the brain. The release of EH is often gated so that it occurs only during a circadian window and accounts for the circadian molting activity of many insects. Its release forms a feedback loop with ETH and as levels of both increase, the expression of preecdysis behavior continues. Eclosion hormone also initiates the release of the **crustacean cardioactive peptide** (CCAP) from the cells of the ventral ganglion that inactivates preecdysis behavior and together with EH activates ecdysis behavior. CCAP is responsible for triggering the motor patterns for the completion of ecdysis behavior. The hormone is also involved in the plasticization of the cuticle that must occur for it to stretch immediately following ecdysis and for the release of **bursicon** that causes cuticular tanning (see Chapter 5). The ability to engage in the stereotyped ecdysial behaviors is acquired and lost at every instar, assembled by ecdysone signaling toward the end of each of the instars by the initiation of gene expression. Once ecdysis takes place, the central nervous system loses its sensitivity to ETH until the next ecdysone peak.

ENDOCRINE CONTROL OF MOLTING

The molting cycle is also initiated and regulated by the endocrine system, and molting occurs as a result of the coordinated activity of all the epidermal cells in the insect, with hormones providing the means by which this coordination occurs. The basic hormonal mechanism is identical whether the molt is larval to larval, larval to adult, larval to pupal, or pupal to adult. What does differ during metamorphosis is the nature of the cuticle that the insect molts to and that is regulated by the presence or absence of juvenile hormone. That mechanism by which a molt results in a different kind of cuticle is discussed in the next section.

The trigger for molting generally correlates with some indicator of growth during the instar. In the few insects that have been studied, PTTH is secreted when a critical size is attained or when stretch receptors are triggered after a large meal is ingested. The PTTH then stimulates the release of ecdysone from the prothoracic glands, which is converted to 20-hydroxyecdysone (20HE), the active molting hormone. PTTH release is governed by a photoperiodic clock and takes place only during circadian gates when the growth-related stimulus occurs. The 20HE circulates in the hemolymph and activates the epidermal cells beginning with apolysis until ecdysis to begin the cycle of epidermal cell division and the synthesis of the new cuticle. The presence of ecdysteroid receptors and their particular varieties or isoforms in the cells at various stages of their developmental programs also determines whether and how the cells will respond to the hormone.

Insects that have been fed optimally ordinarily undergo their molts at fairly predictable times. The ultimate trigger for molting has only been established for

a few insects and appears to be related to the capacity of the nervous system to monitor growth and release hormones appropriately. The blood-sucking bug, *Rhodnius prolixus*, takes a large blood meal once during each instar and then molts to the next instar within days afterward. The insect does not molt in the absence of a blood meal or even when small blood meals are ingested that fail to produce enough abdominal distention. A large blood meal is necessary to trigger abdominal stretch receptors that cause the brain to release PTTH and initiate the molt. Larval *Oncopeltus fasciatus* milkweed bugs initiate a molt when they reach a critical weight, which is presumably monitored by stretch receptors that are activated when growth expands the abdomen.

ENDOCRINE CONTROL OF GROWTH

Multicellular organisms develop their tissues and organs to a size and proportion that is appropriate with environmental and nutritional conditions by precisely regulating the growth of cell size, cell number, and the synthesis of new proteins. Endocrine signals are necessary to orchestrate this growth among tissues so that all organs grow proportionally to each other. Both cell size and cell number appear to be controlled by separate mechanisms and respond independently to environmental variation and gene expression. Whereas the control of molting and developmental transitions involves determining when the insect attains a critical size for the release of PTTH and subsequent ecdysone secretion, the major regulator of growth and metabolism during an instar is the insulin signaling pathway.

Insulin is a member of a larger peptide family that includes several **insulin-like peptides (ILPs)** and **insulin-like growth factors (IGFs)**. Insects and vertebrates show a striking conservation of the insulin signaling pathway. Insulin is well known as a regulator of carbohydrate metabolism in vertebrates and also has effects on embryonic and postembryonic growth. Vertebrates have two related receptors, insulin and IGF-1, that are involved in the regulation of metabolism and growth, respectively. In *Drosophila*, one receptor, **InR**, regulates both processes, and many of the other pathway components have vertebrate homologs. The single InR in *Drosophila* is expressed during embryogenesis and during larval growth in cells of the nervous system and imaginal discs, based on nutrient availability. Loss-of-function mutations in components that transduce the signal in insects decrease cell size, and their overexpression increases cell size. Mutant InR flies share similarities with wild-type flies that are maintained under starvation conditions. The first ILP to be identified in insects was bombyxin, or small PTTH, discussed in Chapter 1, and many ILPs from a variety of insects have since been reported. The *Drosophila* genome bears at least seven ILP genes, expressed by the medial neurosecretory cells of the brain and the corpora cardiaca. Their temporal expression is increased by hemolymph carbohydrate levels

and decreased by starvation, and in *Drosophila* larvae, they have also been shown to regulate JH and ecdysone production by the ring gland (Figure 2.22).

The insulin signaling pathway in insects thus links metabolism and growth with the availability of nutrients. When nutrients are abundant, the pathway is activated as ILPs released from the brain bind to the InR on specific cells and initiate a cascade of events that, among others, activates **protein kinase B** (PKB), also referred to as **serine-threonine protein kinase** (Akt) (Figure 2.23). **PI3 kinase**, activated by the **insulin receptor substrate** (IRS), catalyzes the addition of a phosphate group to phosphoinositides and increases levels of **phosphatidylinositol 3,4,5-triphosphate** (PIP3) that regulates the Akt activity. Akt in turn affects gene expression by influencing a number of transcription factors

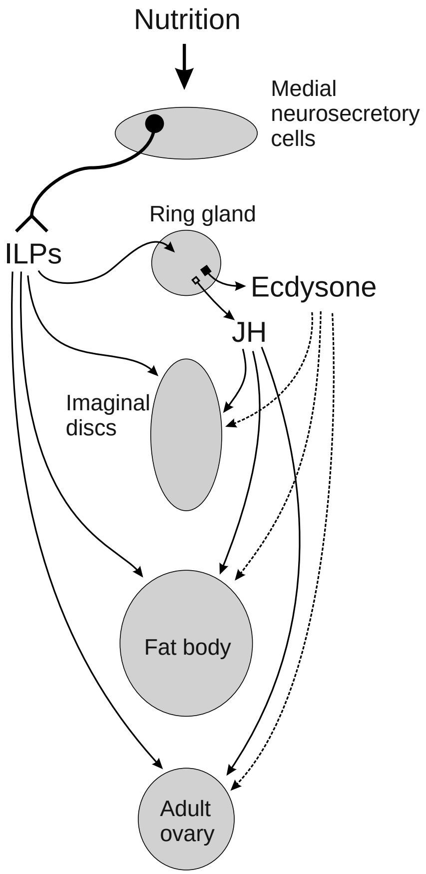

FIGURE 2.22. The effects of insulin-like peptides (ILPs) on growth and reproduction. Nutritional state, established by hemolymph carbohydrate levels, regulates the expression of the ILP genes. The ILPs regulate hormone release and the growth of other cells.

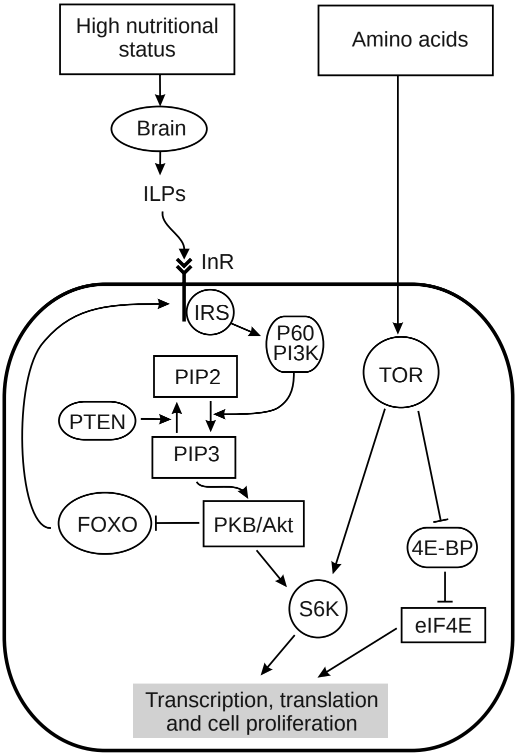

FIGURE 2.23. Cellular pathways of insulin and TOR signaling.

along with the **S6-kinase** (S6K) that regulates the S6 protein of ribosomes. Components that antagonize the pathway include the lipid phosphatase **PTEN** (phosphatase and tensin homolog) that reverses the effects of PI3 on PIP3.

Another related signaling pathway responds to amino acid levels. The **Target of Rapamycin** (TOR) protein responds positively to the presence of amino acids and alters gene transcription and translation by activating S6K (Figure 2.23). It was initially identified in yeasts that were resistant to the antifungal drug rapamycin. TOR inactivates the **4E-binding protein** (4E-BP) that normally binds to and inhibits the **eukaryotic initiation factor 4E** (eIF4E). The resulting dissociation of 4E-BP from the dimer allows eIF4E to participate in ribosome complex formation and translation.

When nutrients are scarce, the insulin pathway is inactivated and growth is inhibited. The presence of the receptor depends on these nutrient levels; InRs increase with limited nutrient availability and decrease with high nutrients. The fluctuation in receptor numbers is regulated by the fork-head box-containing protein (**FOXO**) transcription factor, with high nutrient levels dampening

FOXO activity by causing its retention in the cytoplasm, thereby reducing levels of InR and IRS. With low nutrients, FOXO is allowed to enter the nucleus and promote factors that inhibit cell growth, but at the same time sensitizing the pathway by activating InR.

Those cells destined to proliferate express the InR gene responsible for the insulin receptor, and the ILPs bind to the InR on these cells, controlling proportional growth within the organism and allowing organs to grow to a fixed size. How ILP signaling detects the availability of nutrients is not yet understood. The insulin pathway ultimately controls the levels of protein synthesis and the proliferation of imaginal disc cells to form the adult structures. The overall duration of this growth period is punctuated by ecdysone secretion that counteracts the action of insulin and determines when the transition to the subsequent instar occurs. Ecdysone also allows FOXO to enter the nucleus, altering insulin signaling to inhibit further organismal growth during the molt, and reduces PI3K signaling in the fat body. Insulin signaling additionally modulates JH synthesis by affecting the allatotropins that stimulate JH production. The growth of one organ or tissue can be affected by another neighboring organ or tissue, suggesting that there is a competition for resources that may be mediated by growth factors. These interactions between insulin, ecdysone, and JH are still rather sketchy and only begin to describe the complexity of hormonal signaling that is responsible for growth.

ENDOCRINE CONTROL OF METAMORPHOSIS

Metamorphosis arose once from a hemimetabolous ancestor about 300 million years ago. The type of cuticle that is synthesized during a particular developmental stage, or that is characteristic of a certain region of the integument, depends largely on the proteins that are produced that give the cuticle its specific character and the timing of transcription factors that control their production. Thus, insect metamorphosis is a function of gene expression by epidermal cells and the temporal pattern of their protein synthesis. In contrast to a larval to larval molt where the new cuticle produced is the same as the old one, a larval-to-adult molt in exopterygotes or the larval-to-pupal and pupal-to-adult molts in endopterygotes produce new cuticles that have different properties and contain different proteins. As in a larval-to-larval molt, ecdysteroids still trigger apolysis and the activation of the epidermal cells, but a metamorphic molt also requires the absence of juvenile hormone to reprogram the epidermal cells so they may produce the next stage-specific proteins. A molt that occurs in the presence of JH results in the same type of cuticle being formed, but in the absence of JH the epidermal cells become reprogrammed to produce the cuticle of the next instar. JH appears to act as a status quo agent, preventing epidermal cells from changing their pattern of protein synthesis in the presence of

ecdysteroid. JH has an all-or-nothing effect on metamorphosis: if it is present when target cells are sensitive, the cells fail to change their developmental programs. This sensitivity results from the presence of developmentally appropriate hormone receptors.

The heterodimeric ecdysone receptor complex consists of the ecdysone receptor (EcR) and ultraspiracle (USP). Both EcR and USP occur as multiple isoforms with functional differences, and the profile of isoforms is reflected in the metamorphic stage of the insect. The JH receptor has yet to be identified, but several candidates have been proposed. USP binds weakly to JH, but it does not provide the high-affinity binding that is usually characteristic of hormone receptors. Another candidate is the product of the *Methoprene-tolerant* (*Met*) gene because *Met* mutants in *Drosophila* are resistant to the juvenilizing effects of JH.

More is known about the transcription factors that are present during development. A key transcription factor in holometabolous development is the product of the *broad* (*br*) gene, which is expressed only during the larval-pupal transition of holometabolous insects where it activates pupal-specific genes and directs pupal development. In contrast, *br* is expressed during embryogenesis and throughout the early immature stages of the hemimetabolous milkweed bug, but it is absent during the final larval instar and the adult (Figure 2.24). The metamorphosis of holometabolous insects may have in part been a consequence of this change in *broad* expression. The expression pattern in the milkweed bug is responsible for the differential growth between its larval instars, but it has been more restricted to the last instar of holometabolous insects to direct the changes during metamorphosis.

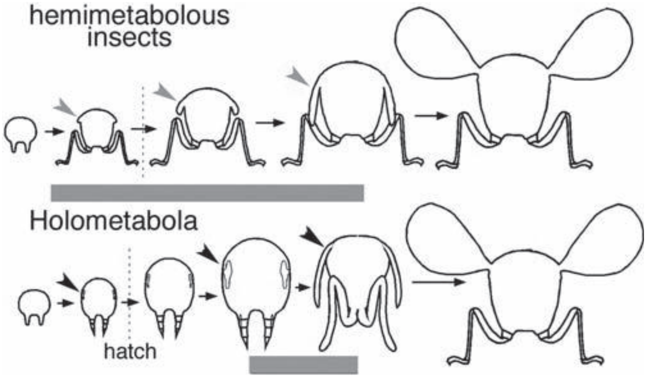

FIGURE 2.24. Proposed role of the broad transcription factor in insect metamorphosis. The images represent cross-sections of the second thoracic segment. Hatching is indicated by the dashed line and arrows indicate wing imaginal discs. The horizontal bar represents *broad* expression, which in hemimetabola occurs throughout the larval stages. In holometabola, *broad* is only expressed during the larval-pupal transition. Reprinted with permission from Erezyilmaz et al. (2006). *Proceedings of the National Academy of Sciences, U.S.A.* 103: 6925–6930.

The JH titer of holometabolous insects declines during the last larval instar, allowing PTTH to be released. This is followed by an increase in ecdysteroid titers as the PTTH activates the prothoracic glands. PTTH release is only inhibited by JH during the last larval instar and may be a mechanism to prevent the production of the molt-associated hormones until all JH has been cleared from the hemolymph. The last larval instar of holometabolous insects is characterized by a series of ecdysteroid releases, the first one being small and occurring in the absence of JH. This absence of JH in the presence of an ecdysteroid peak changes the commitment of the epidermal cells so that they will produce a pupal cuticle when a second increase in ecdysteroid titers occurs. The critical periods for the presence or absence of JH are associated with periods of juvenile hormone sensitivity of the epidermal cells (Figure 2.25).

As with the decision about when to molt, the decision of when to undergo metamorphosis is ultimately determined by the insect's ability to estimate its own size and its developmental arrival in the last larval instar. In the larval lepidopteran, *Manduca sexta*, two indications of size are used. One indicator is the size of the sclerotized head capsule, and the other is the weight of the developing larva. The size of the head capsule tells the larva in which instar the metamorphic molt will occur. Once that recognition occurs, the weight within that final larval instar determines the time during the instar that the metamorphic process begins. The mechanism of how the larva is able to measure these two indicators is not yet known, but it may again be related to stretch receptors or perhaps to the interactions between insulin and ecdysone signaling, based on the size of the prothoracic gland or the degree of nutrient storage.

Whether the larva behaves developmentally as if it were in its last larval instar depends on whether the width of the *Manduca* head capsule is greater than 5 mm. If this width is less than 5 mm, no matter what the developmental or nutritional history of the larva, it will not become a pupa at its next molt and will instead molt to an extra, or sixth larval instar. Thus, there is a threshold size that is required for metamorphosis in that all larvae that enter the last instar with a head capsule width of at least 5 mm will metamorphose during that instar (Figure 2.26).

Several physiological changes occur once the *Manduca* larva is in what has been determined to be its last larval instar. During this last instar, as will be discussed later, the imaginal discs are committed to the production of pupal cuticle. A major change, however, involves the development of sensitivity to JH. Unlike in earlier instars, the brain is no longer able to secrete PTTH in the presence of JH. Similarly, the prothoracic glands cannot respond to PTTH if it is released in the presence of JH. Because during the last instar both the brain and prothoracic gland develop this sensitivity to JH, neither PTTH nor ecdysteroid can be released, and a molt initiated, until all circulating JH has been cleared from the insect. The attainment of a weight of 5 g during this last instar correlates with the development of these events and, presumably, the failure of the corpora allata

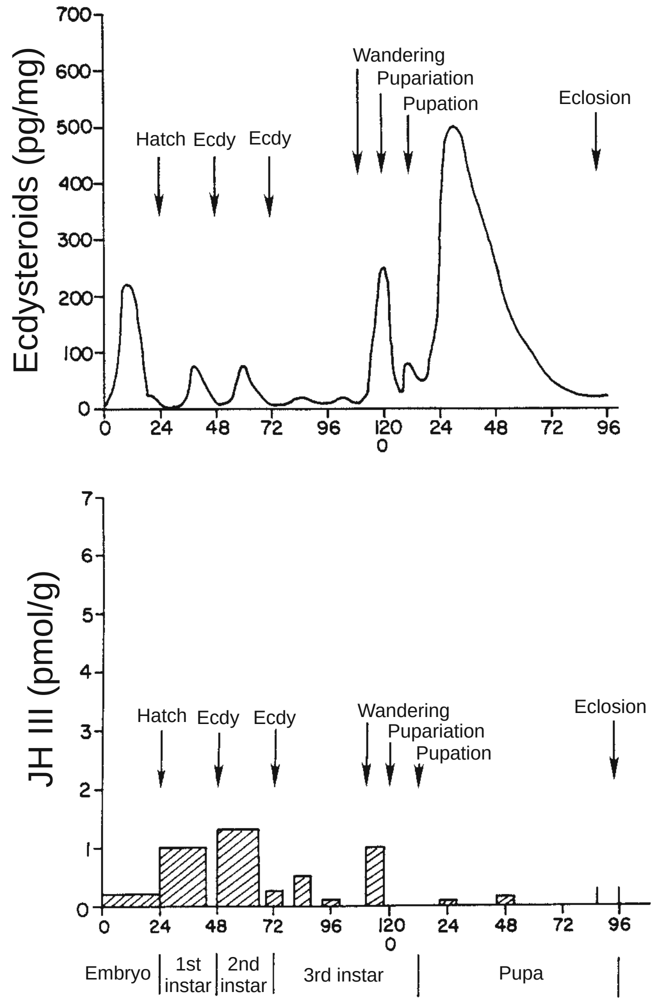

FIGURE 2.25. The correlation of hormone levels with developmental events. (Top) Ecdysteroids. (Bottom) Juvenile hormone III. Reprinted with permission from Riddiford, L.M. 1993. *The development of* Drosophila *melanogaster*, vol. 2, pp. 899–939. Copyright Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

to continue to secrete JH once the insect reaches this weight. After reaching a weight of 5 g, the last instar larva takes about a day to clear all JH from its system, and in the absence of the inhibition by JH, the brain secretes PTTH during the next allowable circadian gate that leads to a metamorphic molt. If PTTH release is inhibited during the circadian gate, its secretion is delayed until the next circadian gate and the larva continues to feed and grow to attain an even greater size. The ultimate size of the adult is thus determined by several

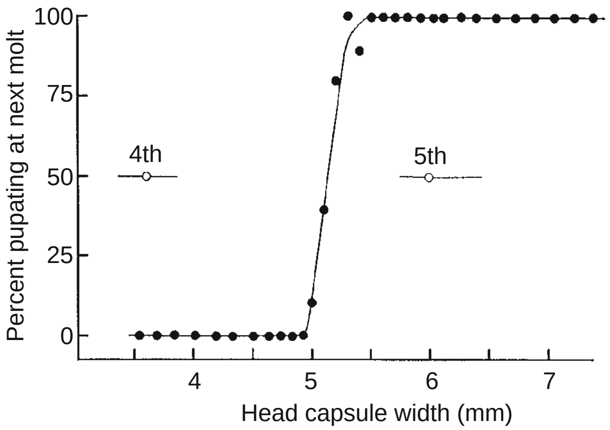

FIGURE 2.26. The relationship between the size of the *Manduca* larva and its tendency to pupate and undergo metamorphosis. By manipulating diet, larvae of a wide range of head capsule sizes can be produced. Those larvae with head capsules greater than 5 mm tend to pupate at their next molt, while those with head capsules less than 5 mm tend to first molt to another larval instar. The horizontal bars show the means and ranges of head capsule sizes for fourth and fifth (last larval instar) instar larvae. From Nijhout (1975). Reprinted with permission.

factors, including the critical weight that is related to the disappearance of JH, the timing of the circadian gate for PTTH release, the rate of growth during the last larval instar, and the size the larva attains during its final instar.

METAMORPHOSIS AND THE RADICALLY CHANGING CUTICLE

Whether they be endopterygotes or exopterygotes, when insects molt from one larval stage to another a new cuticle is produced that is similar to the one being replaced. In contrast, the molt to an adult is associated with a cuticle of a very different nature and often completely different exoskeletal structures. In some insects, such as Lepidoptera, the epidermal cells secrete the larval cuticles as well as the different pupal and adult cuticles, all of which have characteristic proteins and overall patterns. In other insects, such as the higher Diptera, most of the larval epidermal cells are completely replaced by imaginal discs that are epidermal cells that remain undifferentiated during the larval stage but form the various structures associated with the adult, including wings, legs, mouthparts, and the cuticle of the body wall. Thus, there are two approaches to the changing pattern at metamorphosis: larval epidermal cells can change their commitment and alter their developmental secretions to produce various cuticular proteins or be replaced by new cells that form the radically different structures.

Changes in Cuticle Commitment

The ability of larval epidermal cells to change their pattern of secretion and produce a new pupal cuticle depends on the hormonal conditions during the larval-pupal molt and the sensitivity of target cells to the hormones. The windows of sensitivity depend on the presence of cell receptors that are able to recognize and bind the hormones. During the larval-pupal transformation, a small peak of ecdysteroids occurs when the levels of JH are reduced. This is the first time during the life of the insect that cells are exposed to ecdysteroids in the absence of JH. The exposure to ecdysteroids without JH changes the commitment of the cells so when they are next exposed to a higher concentration of ecdysteroids shortly afterward, they produce a pupal cuticle. The general rule they subscribe to is this: if JH is present with ecdysone, continue the current developmental program; if JH is absent and ecdysone is present alone, go on to the next developmental state (Figure 2.27). Following pupal commitment, a mitotic wave with cell proliferation occurs in the pupally committed epidermal cells.

We have already seen (Chapter 1) that ecdysteroids are able to induce puffing at specific sites on the chromosome. The puffing represents mRNA synthesis, and a sequence of gene activation was derived from the sequence of puffing. Many of the early genes induced by ecdysone produce transcription factors that can activate or inactivate other late genes. At metamorphosis, these ecdysteroid-induced transcription factors change, and the new transcription

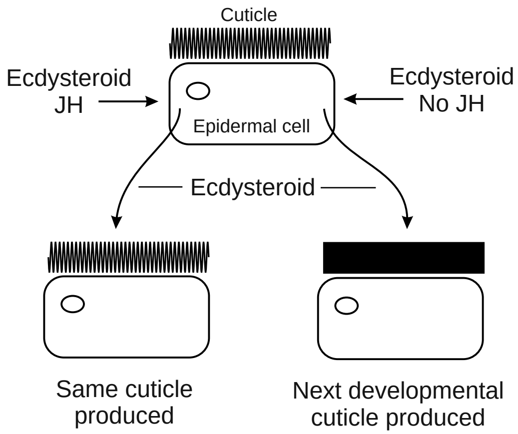

FIGURE 2.27. The mechanism of JH action. A molt is triggered when ecdysteroids act on the epidermal cells. If JH is present during a critical period during which time JH receptors are present on the cell, the epidermal cells produce the same cuticle when they molt. If JH is absent during this critical period and the receptors go unfilled, the epidermal cells produce the next developmental cuticle that they have been genetically programmed to synthesize.

factors induced by ecdysone in the absence of JH may activate the new genes and produce the structural proteins characteristic of the next developmental stage.

Imaginal Disc Development

Metamorphosis in holometabolous insects involves an extensive remodeling of the larva to form the adult. Cells that constitute some larval structures die, whereas other cells are able to persist and synthesize the new products that are characteristic of the new instar. Because adult reconstruction may be beyond the ability of some cells, the new structures are formed from special epidermal cells whose growth is suppressed during larval life and that have no functions in the survival of larvae. These cells are often grouped into distinct imaginal discs or more loosely grouped clusters of histoblasts. Imaginal cells are not unique to holometabola as some hemimetabola also have imaginal histoblasts that replace larval cells. They are employed to different degrees within the holometabola. In higher Diptera and Hymenoptera, the adult epidermis is completely derived from epidermal cells, whereas in some Lepidoptera and Coleoptera, parts of the epidermis may be derived from discs and some from larval epidermal cells that survive metamorphosis.

Most of what we know about imaginal discs comes from work with *Drosophila*. Different discs form the different body parts of the adult fly, each of which contributes to the formation of a different structure. The adult head and thorax are formed from 20 discs, and the abdomen is derived from about 50 histoblasts plus one imaginal disc that gives rise to the adult genitalia. Each of the discs differentiates into a variety of cell types and organs that constitute the adult structures (Figure 2.28). The wing disc, for example, contains about 50,000 cells at maturity that give rise to the structures that make up the wing as well as the portions of the thorax to which it is attached.

The imaginal discs arise as invaginations or thickenings of the ectoderm during late embryogenesis. Their developmental fates are determined in the late blastoderm stage. During larval development, the cells of the discs undergo division and increase in size, attaining maturity during the last larval instar. A mature disc contains columnar epithelial cells surrounded in part by a **peripodial membrane** and attached to the larval epidermis by a stalk (Figure 2.29). A contractile belt of actin and myosin is also present. At the end of the last larval instar, the cells switch from proliferation to differentiation, perhaps after they have undergone a minimum number of cell divisions, and disc elongation and eversion is mediated by changes in cell shape and alterations in cell attachments that are driven by the actin-myosin belts. Cell rearrangement may occur with the expression of new gene products such as the **cadherins** that are calcium-dependent adhesion molecules that function in intracellular adhesion. The eversion causes

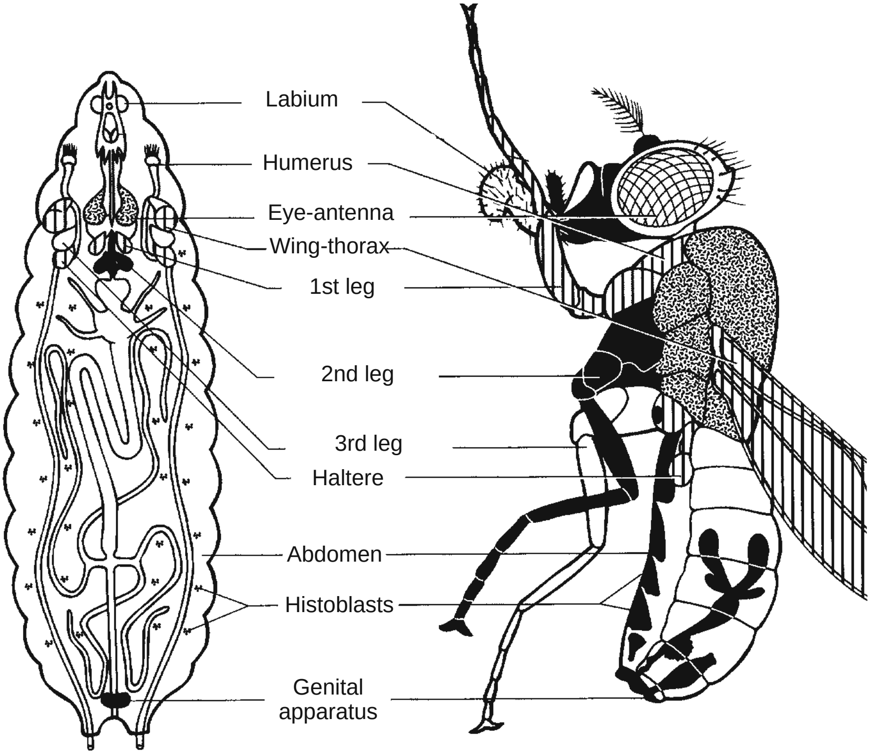

FIGURE 2.28. The imaginal discs of a larval *Drosophila* (left) and the corresponding structures in the adult (right) to which they give rise. From Nothiger (1972). Reprinted with permission.

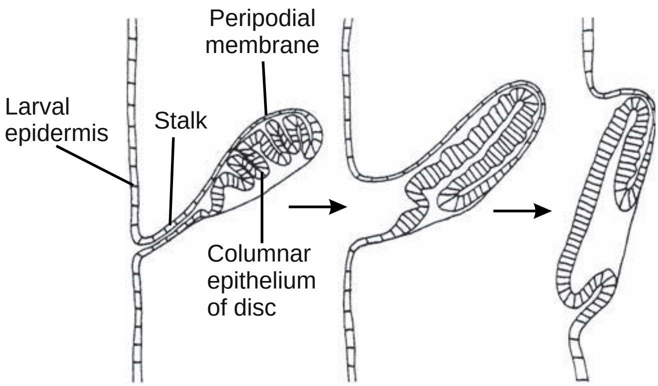

FIGURE 2.29. The development of a *Drosophila* imaginal disc from its attachment by a stalk to its integration into the integument. From Milner and Bleasby (1985). Reprinted with permission.

the apical surface of the disc to become positioned on the outside of the epithelium. The cells then spread from each appendage and fuse with the cells from other discs to form the continuous epidermis of the body wall. Cuticle formation and disc morphogenesis occur at different times, with elongation and eversion possible only when the epidermal cells are not burdened with the overlying cuticle. The ungrouped imaginal histoblasts proliferate during metamorphosis, replacing the larval epidermis as they spread.

Disc development may occur early during embryogenesis or later during the last larval instar. The early-forming discs, such as the wing discs of Lepidoptera, are sequestered during embryogenesis and grow during the larval stages and finally differentiate during the last larval instar. In more primitive holometabola, some adult structures are derived from late-forming imaginal discs derived from clusters of epidermal cells that still retain the potential to form the discs during the last larval instar. The eye imaginal disc of *Manduca* is an example of a late-forming disc that is part of the epidermis of the head capsule until the last larval instar. The expression of the Broad transcription factor reflects the change of commitment in the disc cells.

Disc development and the metamorphosis that results are regulated by both JH and ecdysteroid. JH is present in *Drosophila* during the early larval stages but decreases during the third instar, with a brief peak just before pupariation. This presence of JH during the larval stages allows the cells in the discs to proliferate without differentiating. During this time, JH prevents the discs from responding with a change in commitment when ecdysteroid is present. Differentiation of the discs occurs after pupariation and is the result of high ecdysteroid levels in the absence of JH. The evagination of the disc is followed by the deposition of new cuticle, which only occurs once ecdysteroids decline. A shift from the secretion of exocuticle to endocuticle results from a subsequent small peak of ecdysteroid that occurs slightly later. In the late-forming discs, JH affects two different phases of disc growth: the initial formation of the discs and their later differentiation into a complex structure from the simple epithelial sac. This JH-regulated morphogenesis occurs independently of ecdysone, but nutrient-dependent signals are also involved.

The maturation of adult genitalia originates in a common genital disc in both *Drosophila* sexes. The single genital disc that is employed by both sexes gives rise to the entire set of internal and external components, plus the anus, that are recruited from different segments. Sex determination in *Drosophila* is ultimately based on the ratio of X chromosomes to autosomes, with XY males having a ratio of 1:2 and XX females with 2:2. This X:A ratio implements the state of activity of a cascade of sex-determination genes that directs the particular form of genitalia characteristic of that sex. The cascade exists as a parallel pathway to the one that leads to *fruitless*, a gene governing sexual behavior (Chapter 5). The last gene in this sex determination cascade is *doublesex* (*dsx*), whose product is a transcription factor that is contingent on the sex that has been established by the

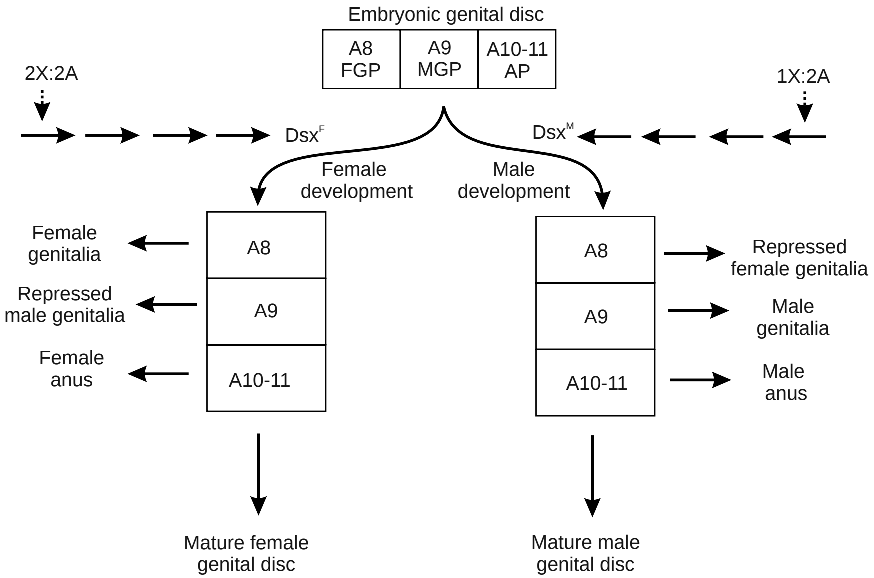

FIGURE 2.30. The development of male and female genitalia in *Drosophila* from the single genital disc consisting of a female genital primordium (FGP), a male genital primordium (MGP), and an anal primordium (AP). A series of steps based on the ratio of X chromosomes to autosomes results in the synthesis of either the Dsx^m or Dsx^f proteins that lead to either male or female disc development. For female disc development, the genital primordium on abdominal segments 8 (A8) and 10–11 (A10–11) mature, while for male disc development A8 is repressed and A9 develops with A10–11.

X : A ratio. In the development of females, the 2X : 2A ratio causes the *dsx* gene to produce the Dsx^F protein, promoting female genital disc development. For developing males, the 1X : 2A ratio causes the *dsx* gene to produce the Dsx^M protein that leads to male development of the disc (Figure 2.30). The mature disc is formed by the fusion of cells from abdominal segments 8 to 11, with segment 8 bearing the female genital primordium, segment 9 the male genital primordium, and segments 10 and 11 the anal primordium. Intersexes bear male and female genitalia when both Dsx^M and Dsx^F proteins are produced by the same individual.

Metamorphosis, Cell Reorganization, and Cell Death

The often radical changes that occur during metamorphosis allow the life stages of the holometabolous insect to occupy radically different ecological niches. The terrestrial larval stage is mainly focused on feeding and growth and spends its time crawling. In contrast, the winged adult is occupied with reproduction and

dispersal and has a completely different diet. These changes are made possible not only by the appearance of novel structures that arise from imaginal discs during development but also by the disappearance of many larval tissues and their capacity to change their structure and function in the adult.

The prothoracic glands produce the ecdysteroids that trigger the molting process and are essential for the larval stage to undergo its periodic molts. However, adult pterygotes no longer molt, and the glands no longer have any function in this stage. They are absent in most adult insects because of their programmed degeneration during metamorphosis. In both *Manduca* and *Drosophila*, the glands degenerate during the pupal-adult transition as a result of their exposure to the peak of ecdysteroids that they themselves produce in the absence of JH secretion. Similarly, the silk glands that are used by lepidopteran larvae to form the cocoon for pupation begin to degenerate after the first ecdysteroid peak that occurs in the absence of JH during the last larval instar. The larval midgut and salivary gland cells of *Drosophila* undergo a histolysis in response to the peak of ecdysteroid during the prepupal-pupal molt. The reformation of the adult midgut occurs more slowly, with the adult gut forming around the degenerating larval gut, and is preceded by a cessation of larval feeding behavior that may minimize the complications that food in the rearranging gut might create.

The cells of the larval fat body are different from that of the adult in form and function. The larval fat body of *Drosophila* is a lobed monolayer of polytene cells that separate; become depleted of glycogen, lipid, and protein; and subsequently degenerate during the early adult stages. The cell separation results from the production of the proteolytic enzyme cathepsin that is produced by hemocytes in *Sarcophaga*. The adult fat body is arranged in sheets just below the epidermis and develops from precursor cells. Another method of larval fat body restructuring occurs in the lepidopteran *Calpodes*, which is arranged in ribbons one cell thick and reforms in the adult as lobes attached to trachea.

Structural changes in appendages and the body wall also require changes in muscle and nervous innervation. During metamorphosis, the larval muscles of insects can either die or become remodeled. *Manduca* larvae bear abdominal prolegs that aid in crawling, and these structures are not present in the flying adult. The muscles that retract the larval prolegs and allow their movement to occur degenerate on the day after the wandering stage begins. There is a second wave of muscle degeneration that occurs after adult emergence when larval muscles that are also used for early adult ecdysial behaviors disappear. The larval intersegmental muscles of *Manduca* that are also used during adult ecdysis to escape from the pupal skin are delayed in their death until after this event occurs. The timing of the two waves of muscle degeneration in *Manduca* during metamorphosis reflect the delicate balance between the termination of larval functions and the development of adult functions during this period. The remodeling of muscles also occurs. After the degeneration of the contractile apparatus of some larval muscles and the loss of muscle cell nuclei, the remains are used to build

the adult muscles. New muscle attachment sites are created and the remodeled muscle gains a function distinct from its larval predecessor. *Drosophila* embryos have precursors for two sets of muscles that arise from the mesoderm and initially express the gene *twist.* Larval muscles cease their production of twist protein, but the adult muscle cell precursors continue its expression and delay their differentiation. During metamorphosis, most larval muscles degenerate and are replaced by the adult muscles, but some remain and serve as a scaffold for the adult muscle migration and assembly.

The motor neurons that innervate muscles are likely to change during metamorphosis. The increase in ecdysone with molting triggers the deaths of the neurons supplying the larval prolegs, but the delayed death of the intersegmental muscle motor neurons is a result of the declining levels of ecdysone after adult emergence. The presence of the hormone sustains this population of neurons during larval development and the absence of the hormone after the pupal-adult molt causes their decline.

A more fundamental reorganization of the nervous system also occurs during metamorphosis. The nervous system of the newly hatched hemimetabolous insect is essentially complete and does not change substantially during its metamorphosis. Except for a few neural additions to accommodate the appearance of the adult compound eyes, the hemimetabolous larva begins life essentially with an adult nervous system. However, holometabolous larvae differ drastically from adults in locomotion, sensory reception, and behavior, and the way the nervous system couples this new sensory information to novel motor outputs must change radically to accommodate these differences. Holometabolous larvae are responsible for acquiring food and the adults for reproduction and dispersal. Not only must newly formed structures be innervated and the nerves providing the connections to old structures be redesignated or terminated, but the new interneurons that form the connections between the sensory input and motor output must also be created.

The neurons that innervate larval structures destined to disappear in the adult, such as the larval prolegs, die soon after the larval-pupal molt. Other motor neurons that innervate larval muscles also used during adult emergence die soon after adult development is complete. Approximately half the neurons in the central nervous system of the larva die shortly after adult emergence. Other larval neurons are remodeled and used for other functions in the adult. At metamorphosis, there is a reduced arborization of larval neurons and outgrowths of adult-specific neurons. The pruning back of larval dendrites occurs after entry into the pupal stage, followed by the outgrowth of the adult dendrites with exposure to the rising titers of ecdysone and the absence of JH at this time. Many neurons of the holometabolous adult arise from embryonic neuroblasts that are arrested until metamorphosis and then proliferate to create the interneurons that link sensory and motor nerves. As a result of this developmental arrest, larval holometabola tend to have fewer interneurons in the central nervous system than do

larval hemimetabola, and this relatively simplified system may explain their relatively simple behaviors and the reasons that hemimetabolous larvae are more adult-like.

The death of some neurons during the larval-pupal molt is a function of the hormonal conditions that exist during the last larval instar. Two peaks of ecdysteroid occur, the first of which occurs in the absence of JH and has already been identified as the so-called commitment peak, which is the first time in the life of the organism that ecdysteroids appear without JH. When the neurons that innervate the disappearing larval prolegs are exposed to this commitment peak, they become more sensitive to ecdysteroids and degenerate several days after being exposed to the second larger ecdysteroid peak. The temporal sequence of degeneration of larval cells and their differing responses to ecdysteroids as the trigger for degeneration may be related to the multiple isoforms of the ecdysteroid receptor, EcR. The three EcR isoforms that are currently known to combine with the other nuclear receptor, USP, to form the heterodimer that binds with ecdysteroids are EcR-A, EcR-B1, and EcR-B2. Larval neurons do not bear any EcR receptors until the last larval instar, when EcR-B1 and EcR-A predominate. High levels of EcR-A are correlated with cell death while the presence of EcR-B1 in a cell is correlated with the tendency of neurons to undergo remodeling. The particular EcR/USP/ecdysone complex induces a set of early genes encoding transcription factors that amplify the hormonal signal by regulating the expression of secondary response genes. Early genes include *Broad, E74,* and *E93*. *Broad* and *E74* are expressed after each ecdysone pulse, but *E93* shows a stage-specific expression in the salivary glands of larvae. The consequence of this differential gene expression in different tissues is the tissue-specific response to ecdysone during metamorphosis, when a pulse of the hormone can cause histolysis in one tissue when it may trigger evagination and procuticle growth in another.

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CHAPTER 3

Developmental Systems

Multicellular organisms begin their lives as single-celled zygotes that divide to form groups of cells that show progressive morphological changes. Although they are all genetically identical, successive daughter cells give rise to all the diverse cell types that will make up the multicellular insect. The development of these morphological differences and the generation of cellular diversity is the process of **differentiation**. However, long before the actual differentiation is evident, the developmental fate of the cells is fixed. Although they may not yet show any morphological differences, they have become genetically committed to a particular course of development. The commitment process that establishes the later differentiated state is **determination**. Thus, the nonvisible process of determination is followed by the detectable state of differentiation. These processes begin in the egg and continue during the life of the organism. An organism's **ontogeny** involves the entire process of transforming it into a mature adult from a fertilized egg.

INSECT EGGS

The insect eggshell, or **chorion**, has a number of critical responsibilities. Most important, it must serve as a two-way barrier to prevent the loss of egg contents

to the environment and also minimize the disturbance of those contents by environmental hazards. It does this while remaining flexible enough to pass through a narrow ovipositor, permitting sperm to enter for fertilization, and facilitating the escape of the larva at the termination of embryogenesis. In many aquatic insects, the egg contains special external structures that hold them upright on the surface of the water. Specialized respiratory structures may also branch off the main body of the egg.

The embryo has all its metabolic needs prepackaged inside the egg, but one critical component, oxygen, is lacking and must be acquired from the outside. This presents a challenge in design because given the small size of insect eggs and the resulting high surface-to-volume ratio, any gaseous exchange by the egg brings with it an enormous potential for water loss. The complex structure of the insect chorion is a compromise between facilitating oxygen gain and minimizing this loss of water. Males of the giant water bug, *Belostoma*, address this compromise by permitting females to oviposit on their backs and then properly positioning themselves at the air-water interface to ventilate the eggs while preventing them from drying or drowning. Because higher temperatures increase the metabolic rate of the embryo within the egg but not the diffusion rate of oxygen, larger insect eggs, such as those of *Manduca*, can actually be oxygen limited at higher temperatures. The larger insect eggs characteristic of the late Carboniferous may have been possible because they took advantage of the higher concentrations of oxygen (up to 35% compared to 20% today) coupled with cooler temperatures.

Among the other critical needs are various protective strategies. The physical disruption of egg membranes can be repaired internally, making it possible to experimentally inject substances into the egg. Heat-shock proteins that are present can protect other developmental components from denaturation by heat or other environmental stressors. The gradients of morphogens that guide and regulate embryonic development maintain their stability even under changing genetic and environmental circumstances.

Holometabolous development was a major advance that is considered to be a key to the success of insects, allowing the adults and immatures of a species to occupy divergent habitats and thus reduce the competition between them for resources. This divergence of habitat preferences also reduced the opportunities for parental investment and made its development more costly, as adults no longer routinely occupied the environments of their offspring. Because there was an increased need for eggs to survive on their own under these circumstances, the design of the egg was of considerable importance. With the evolution of ovipositors, females were able to deposit their eggs in concealed locations as well as novel habitats that were unavailable to other animals. It might be said that holometabolous development could only have occurred once the egg and egg-related structures had been sufficiently altered to allow survival under varied aquatic and terrestrial conditions. There are many exceptions, but ametabolous

and hemimetabolous insects generally have larger eggs with more yolk than do holometabolous insects.

Egg Membranes

The eggshell, or chorion, is a complex of several layers (Figure 3.1). It is synthesized within the ovariole by the **follicular epithelium** that surrounds the oocyte and begins once **vitellogenesis**, the uptake of yolk proteins, has been completed. In the silkworm moth, *Antheraea polyphemus*, approximately 10,000 follicle cells surround the oocyte in a monolayer and secrete the complex layers of the chorion, only to degenerate once the chorion has been completely

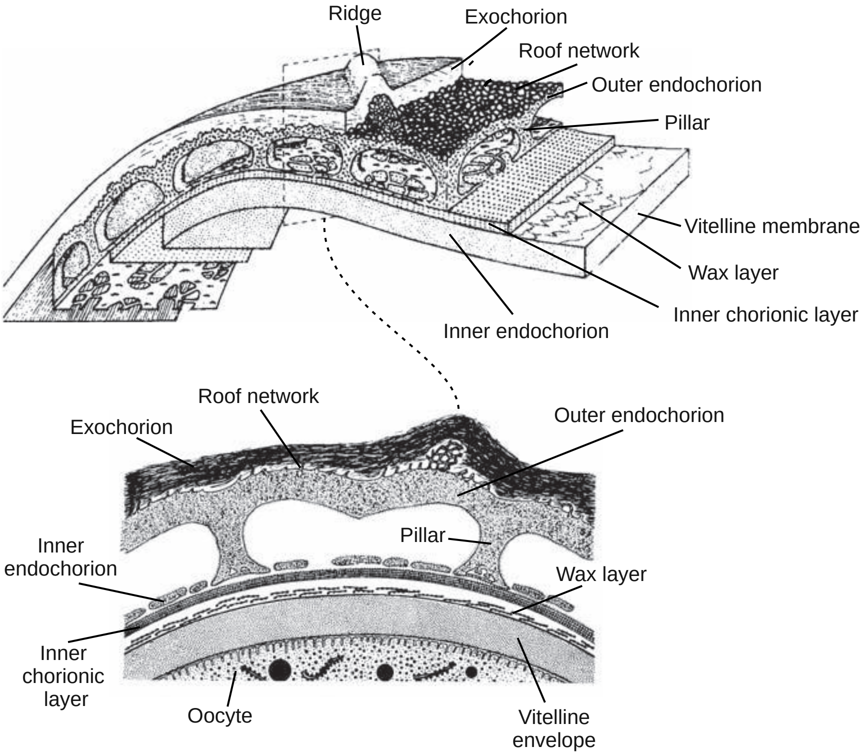

FIGURE 3.1. Cross-sectional representations of the chorion of *Drosophila melanogaster*. The figure below is a two-dimensional cross section of the region shown by the dashed square in the figure above. Reprinted from Margaritis, L.H., and M. Mazzini. 1998. *Microscopic anatomy of invertebrates*, vol. 11C, pp. 995–1037. This material is used by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

formed. The follicle cells often leave a species-specific imprint on the surface of the egg. Because these follicle cells surround the oocyte and direct their products inward, the inner layers of the chorion are secreted first with subsequent layers formed either by **apposition** of newly synthesized layers or by the **intercalation** of components into previously formed layers. Their synthesis may occur in an orderly progression according to the sequential activation of chorionic genes, but there is considerable movement of proteins within the chorion once they are secreted. Relocation of proteins to the vitelline membrane, endochorion, and inner chorionic layer occurs late during oogenesis.

Choriogenesis is a period of intense protein synthesis, and before chorion gene expression the follicle cells undergo several rounds of DNA replication without cell division to increase the DNA content and their synthetic capability. Some of the chorion genes may be additionally amplified to 60 to 80 times above the usual copy number to meet the increased demand for chorionic proteins by enhancing their biosynthetic rate. In *Drosophila,* amplification begins at stage 10B of oogenesis and is completed by stage 11, allowing all the required chorionic proteins to be synthesized within a 5 hour period. The formation of the chorion has only been studied for a handful of insects, and most generalizations are based on *Drosophila melanogaster*, *Antheraea polyphemus*, or *Bombyx mori* models.

The first layer of the chorion to be synthesized by the follicle cells is the **vitelline envelope**, an inner noncellular membrane with a thickness of about 0.3 μm that completely surrounds the oocyte, except at the **micropyle**, the site of future sperm penetration. The envelope is first deposited as plaques that ultimately coalesce over the oocyte membrane. In many insects, a thin **wax layer** ranging in thickness from 5 to 10 nm may then be deposited between the vitelline envelope and the follicle cells to provide protection against desiccation. This layer is the primary waterproofing layer of the egg. The presence of a wax layer has not been identified in all insect chorions.

Above the wax layer are several other chorionic layers that, depending on species, may be formed by the apposition of newly secreted layers or the intercalation of secretions into existing layers. A thin **inner chorionic layer** that consists largely of protein may be secreted above the vitelline envelope. The **early proteins** of the **endochorion** begin to be synthesized after the wax layer is laid down and form a scaffold on which middle and late proteins intercalate. There has been a remarkable conservation of early proteins among insect genera, with a greater divergence in the structures of the middle and late proteins. The endochorion is divided into an **inner endochorion** of about 400 Å in thickness, consisting of a network of pillars, and the **outer endochorion** of approximately 0.2 μm in thickness that creates a roof network. Between the pillars is a meshwork that can trap air, with pores or **aeropyles** that open to the outside. The aeropyles may be grouped in specific areas, such as in the vicinity of hatching lines, and allow the passage of air into the meshwork of the endochorion while

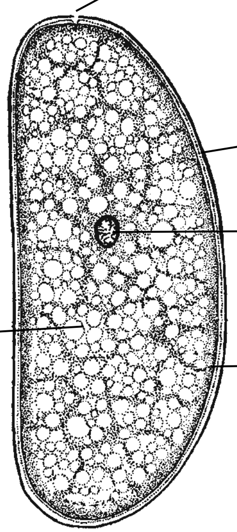

FIGURE 3.2. Cross section of a generalized insect egg prior to fertilization. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

restricting the passage of water. The **exochorion** is a 0.3 μm thick outermost layer that may contain carbohydrate in addition to protein. Indeed, except for the wax layer, the chorion consists primarily of protein that can be cross-linked by the process of sclerotization to stabilize its structure. Many of the chorionic proteins belong to related families of chorion genes that have evolved as a result of the duplication of an ancestral gene.

Some regions are differentiated into more specialized structures. Because the eggshell is so impermeable, there must be a special provision to allow sperm to enter. The **micropylar apparatus** is synthesized by the border cells, special cells dispersed early during oogenesis among the follicle cells. It consists of an opening, the **micropyle**, and a canal that leads to the oocyte (Figure 3.2). The micropyle is sufficiently wide to allow only a single sperm to enter. It may be a protrusion or a depression, containing small holes that penetrate through the chorion and the vitelline membrane to the surface of the oocyte. There may be a single micropyle or as many as 70, and although they are usually located at the anterior end of the egg, they are found on the ventral side in crickets and at the posterior end in termites and scorpion flies.

In addition to the aeropyles that channel air into the chorionic meshwork, other specialized regions of the chorion, such as respiratory appendages, may be present that can serve as a plastron to extract oxygen from water (Figure 3.3).

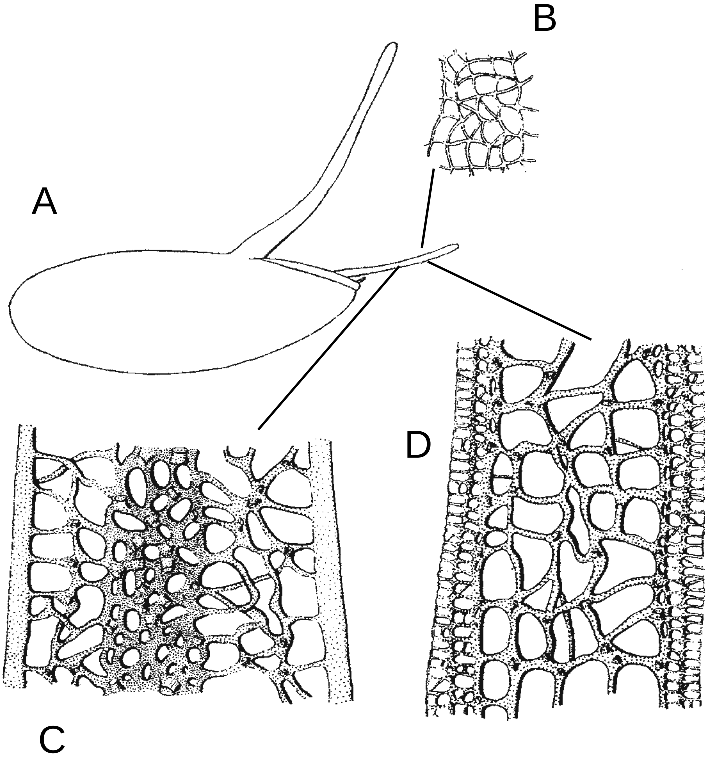

FIGURE 3.3. A. The egg of *Drosophila gibberosa* and its respiratory horns. B. The surface network of the horn at the region indicated. C–D. The meshwork of the respiratory horn seen in optical section. Reprinted with permission from Hinton, H.E. 1960. Phil. Trans. R. Soc. Lond. B 243: 45–73.

These are found both in eggs of aquatic species as well as those terrestrial eggs that may become periodically inundated by water. There may be regions of programmed weakness in the chorion to allow the larva to more easily escape during hatching. The **operculum** is a cap that is surrounded by these hatching regions and opens to allow the larva to exit. In some species, first instar larvae have a special spine, or egg burster, to facilitate the breaking of the chorion.

Pattern Formation within the Oocyte

Given the limited amount of space inside the tiny insect egg, it is somewhat surprising that its internal composition lacks uniformity. There are distinct regions at the anterior and posterior poles, and the cytoplasm immediately beneath the plasma membrane, the **periplasm**, is morphologically different from that of the

interior of the oocyte (Figure 3.2). The micropyle, a channel for sperm entry, is located at the anterior pole where the head of the embryo will develop, and in *Drosophila*, respiratory appendages lie on the dorsal side. Within the egg lie not only the oocyte and the raw materials necessary for its growth but also a complex of materials that specify how that growth should occur. In addition to the protein yolk, lipid, messenger RNA, and maternal organelles such as mitochondria and ribosomes, there are also gradients of protein that result from the transcription of specific genes, all of which are spatially organized to create a pattern that provides the developing embryo with positional information within the egg. Proper development involves a sequential activation or repression of specific genes by gradients of these **morphogens**, and this positional information is crucial for the expression of genetic information at the appropriate time and place during development. Thus, although much of the information that allows the organism to develop correctly resides in the genome, considerable information is also derived from this nonuniform placement of materials in the egg.

Polarity gradients of these materials are established early during oogenesis while the oocyte resides within the female, even before fertilization and oviposition occur. Preformed mRNAs laid down by the mother form an anterior-posterior polarity in the egg established by **maternal effect genes** in the nurse cells of the female parent that are the first to encode various regulatory proteins that diffuse throughout the egg and specify positional information that activates or represses the expression of certain genes later during development.

One of the first morphogenetic gradients in the developing *Drosophila* embryo establishes the cytoskeletal framework on which other spatial patterns are based. Soon after the oocyte differentiates from nurse cells, it migrates to the posterior of the nurse cells where it induces the follicle cells there to adopt a posterior fate instead of the anterior fate that is their default state. The oocyte nucleus serves as a collection site for a maternally derived *gurken* RNA that is translated into a Gurken protein. The Gurken protein passes into the posterior follicle cells; in response, these cells produce a *torpedo* gene product that binds to Gurken and establishes their posterior cell identity. These posterior follicle cells then send a signal back to the oocyte that polarizes its microtubular cytoskeleton and directs the localization of the *oskar* and *bicoid* RNAs to either end of the oocyte, establishing the anterior-posterior axis. The anterior development is directed by *bicoid*, and the formation of the posterior pole plasm where the germ cells are localized and develop is established by *oskar* (Figure 3.4).

Continued nuclear movement similarly establishes dorsal-ventral polarity. The cytoskeletal repolarization subsequently induces the oocyte nucleus to move to the anterior dorsal portion of the cytoplasm where its development continues and sets the dorsal-ventral identities. The Gurken signal carried by the nucleus causes the follicle cells in this area to adopt a dorsal fate by expressing the gene *kekkon* and specifies that side of the oocyte as the future dorsal region, giving rise to dorsal chorion structures such as the respiratory horns. Ventral cells not

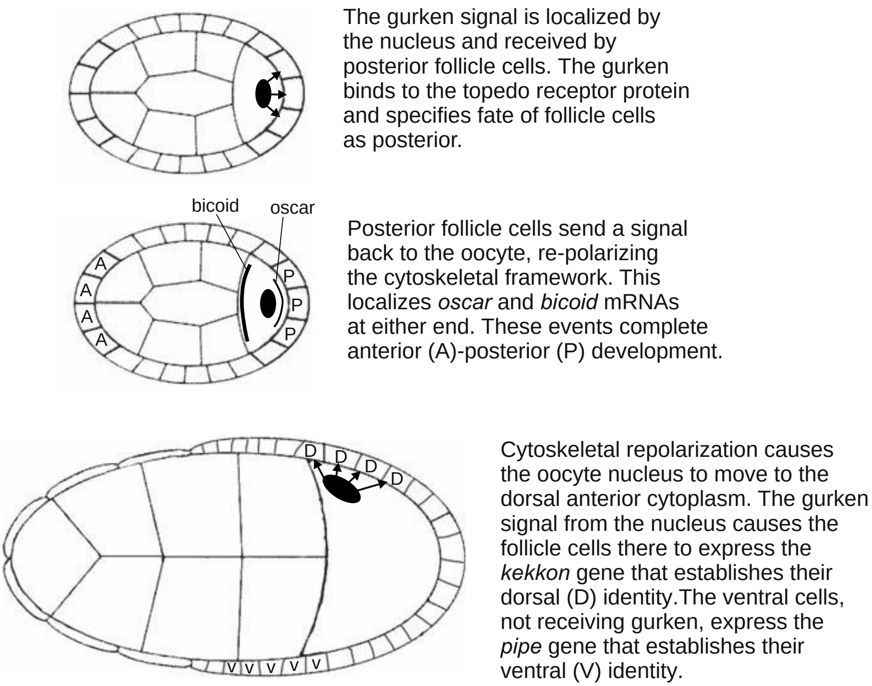

FIGURE 3.4. The establishment of anterior/posterior and dorsal/ventral commitment in follicle cells.

receiving the *gurken* message express the gene *pipe* that establishes their ventral identity. So, the *gurken* message, originating from maternal mRNA, sequentially induces both posterior and dorsal cell fates in the follicle cells even before the egg is laid (Figure 3.4).

What little is known about how the morphogenic gradients regulate the determination of cells paints a picture that is already incredibly complex. Maternal effect genes encode at least four maternal mRNAs that are involved in the subsequent determination of anterior/posterior patterning in the fertilized egg: *bicoid* and *hunchback* mRNAs regulate the development of structures characteristic of the anterior of the egg, whereas *nanos* and *caudal* regulate posterior structures. After fertilization, the *bicoid* mRNA localized at the anterior end is translated, and the Bicoid protein, a transcription factor, forms a gradient along the anterior-posterior axis. Embryos that lack Bicoid protein have no organized head or thorax. The Bicoid protein specifies the anterior of the embryo in two ways. It prevents the posterior determination in anterior regions by binding to and suppressing the anterior translation of the *caudal* mRNA that is normally found dispersed throughout the egg. When present anteriorly, the *caudal* message prevents the proper formation of the head and thorax. Bicoid protein also activates

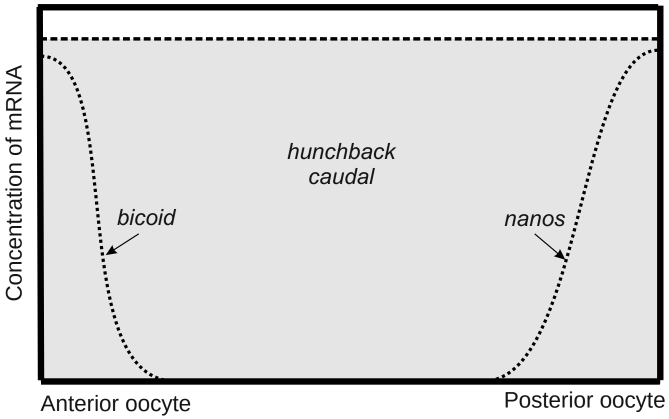

FIGURE 3.5. The gradients of *bicoid*, *nanos*, *hunchback*, and *caudal* mRNA that establish position in the *Drosophila* oocyte.

the *hunchback* gene that specifies anterior structures, and together they specify the anterior pattern.

The maternal *nanos* gene produces mRNA that is localized at the posterior pole of the egg. The Nanos protein represses the translation of any *hunchback* RNA that is present in the posterior region at the same time the *hunchback* is being activated at the anterior by Bicoid protein. If the *nanos* gene product were to be absent, Hunchback protein would be synthesized in other areas and affect the expression of other genes that are required for differentiation of structures in the abdominal region. The distribution of these mRNAs according to this model for development is shown in Figure 3.5.

In *Drosophila*, the maternal gene patterning next regulates the expression of the zygotic genes in certain broad regions along the anterior-posterior axis. These are the **gap genes**, named because mutations in them produce gaps in the segmentation pattern. The gap genes include *giant*, *hunchback*, *Krüppel*, and *knirps*, all of which code for transcription factors. The gradient of Bicoid protein activates the anterior expression of *hunchback*, which then controls the expression of the other genes. The zygotic hunchback protein is produced against the background of existing maternal hunchback.

The concentrations of the gap gene proteins subsequently cause the transcription of **pair-rule genes** that divide the embryo into periodic units. There are 14 of these periodic **parasegments** in *Drosophila,* each of which is an independent developmental unit under the control of specific genes and will develop uniquely into the segments of the mature larva. Each parasegment will include the posterior portion of an anterior segment and the anterior portion of the segment just behind it. The pair rule genes are expressed in stripes and define the parasegments, with some genes, such as *even-skipped*, defining the odd-

numbered parasegments and others, such as *fushi tarazu*, defining the even-numbered parasegments. The expression of pair rule genes depends on the presence of Bicoid protein and the expression of *giant*, *hunchback*, and *Krüppel*, and the transcription factors these code for allow the pair rule genes to set the framework for the development of final segmentation and segment identity.

The ephemeral activity of the pair rule genes activates the transcription of the **segment polarity genes**, so called because mutants have disrupted anterior-posterior polarity. The segment polarity genes are activated within each parasegment, and *engrailed* is expressed at the anterior margins. This expression establishes the boundaries of **compartments**, groups of cells that are under a common genetic developmental control whose descendants never move across the boundary line. The parasegments later give rise to the segments of the larva, and as the anterior region of the parasegment becomes the posterior region of the segment, *engrailed* expression defines the posterior compartment of the segment. Unlike the short expression of the pair-rule genes, *engrailed* expression continues through the embryo to the larva and pupa and is also a homeotic gene, the next class to be described. Other segment polarity genes include *wingless* and *hedgehog*, all of which establish and maintain these parasegmental boundaries that are characterized by their own systems of positional information.

Each of the parasegments is identical but must be transformed into the unique segment that makes up the mature organism by the **homeotic genes**, or ***Hox* genes**. The name homeotic originates from the effects their mutations have on transforming one structure into another, or **homeosis**. Also known as **selector genes**, these specify the segment identity and determine the developmental fate of each of the segments. Without the *Hox* genes, all segments in the insect would look alike. They appear to be universal among multicellular organisms, having been identified in a number of animals from nematodes to chordates. The genes all contain a highly conserved sequence of 180 base pairs called the **homeobox** that encodes a transcription factor that binds to specific DNA regions.

Once the segmental boundaries are established, *Hox* genes determine the developmental pathways within each segment. The *Hox* genes involved in segment identity are organized into two gene complexes on chromosome 3, **Antennapedia** and **bithorax**, with their 3′ to 5′ order on the chromosome reflecting their spatial expression and timing (Figure 3.6). Mutations in these

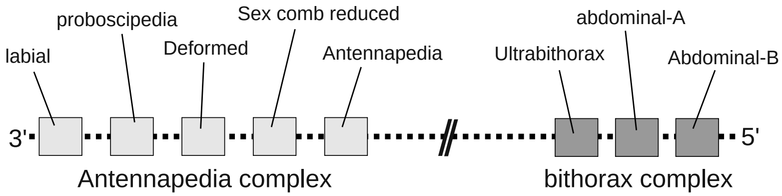

FIGURE 3.6. The Hox gene complexes arranged linearly on chromosome 3 of *Drosophila*.

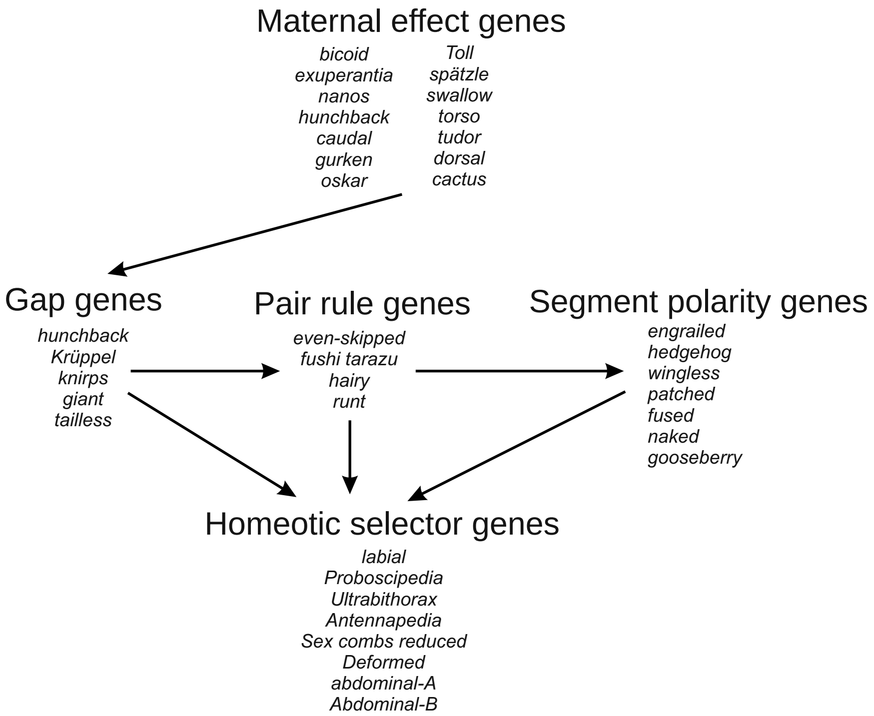

FIGURE 3.7. Genes involved in spatial patterning during *Drosophila* development.

genes can cause bizarre developmental errors, such as antennae growing out of leg sockets, legs growing out of the head where antennae normally develop, or the halteres developing into a second pair of wings. The proteins of the gap, pair-rule, and segment polarity genes interact in the regulation of the *Hox* genes to specify segment identity rather than specifying a particular structure within the segment. For example, the gap gene proteins Hunchback and Krüppel inhibit the *abdominal-A* and *abdominal-B* genes in the head and thorax to prevent them from specifying abdominal characters, and *Ultrabithorax* is activated by concentrations of Hunchback protein at the center of the embryo. *Hox* genes are expressed in the ectoderm and visceral mesoderm but not in endodermal tissues that give rise to the midgut. Figure 3.7 shows the major genes that are involved in the spatial patterning in the *Drosophila* embryo.

EMBRYONIC DEVELOPMENT

The oocyte, which has been arrested in metaphase of the first meiotic division, continues to mature after oviposition occurs. The oocyte completes meiosis

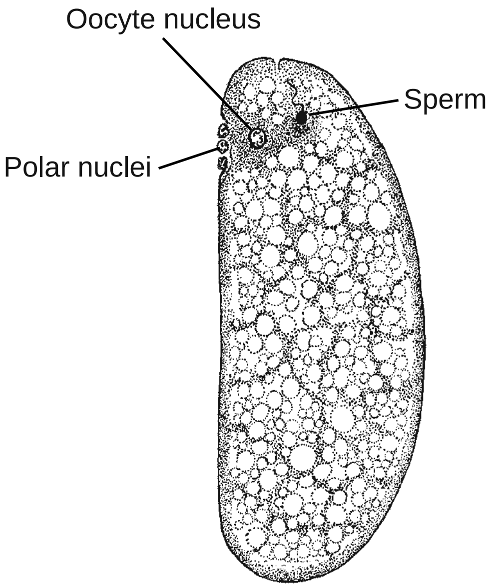

FIGURE 3.8. The generalized insect egg after sperm entry but prior to ferilization. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

shortly after the sperm penetrates the egg and oviposition takes place. This results in a haploid oocyte nucleus and three polar nuclei that inhabit the periplasm at the periphery of the egg (Figure 3.8). The oocyte nucleus, surrounded by an island of cytoplasm, then moves to the interior of the egg where it meets the sperm that has already entered. **Syngamy**, the union of sperm and egg, occurs at the interior. The polar nuclei may accompany the oocyte or degenerate at the periphery. In some insects that reproduce without sperm involvement by **parthenogenesis**, a haploid polar nucleus combines with the haploid oocyte nucleus to restore the diploid number without requiring fertilization by male gametes.

Following the union of the sperm and the egg, the newly formed zygote undergoes **cleavage** within the patterned environment that is present in the egg. Cleavage is the process by which the zygote divides mitotically to parcel out the cytoplasm into other smaller daughter cells. The pattern of cleavage in a particular animal species is asymmetric and determined largely by the amount and distribution of the yolk within the egg cytoplasm. This produces daughter cells that are genetically identical but that differ in their cytoplasmic components. The presence of yolk in the egg tends to inhibit cleavage, so animals that have relatively little yolk are able to undergo complete or **holoblastic** cleavage (Figure 3.9). However, the relatively large amount of yolk in most insect eggs

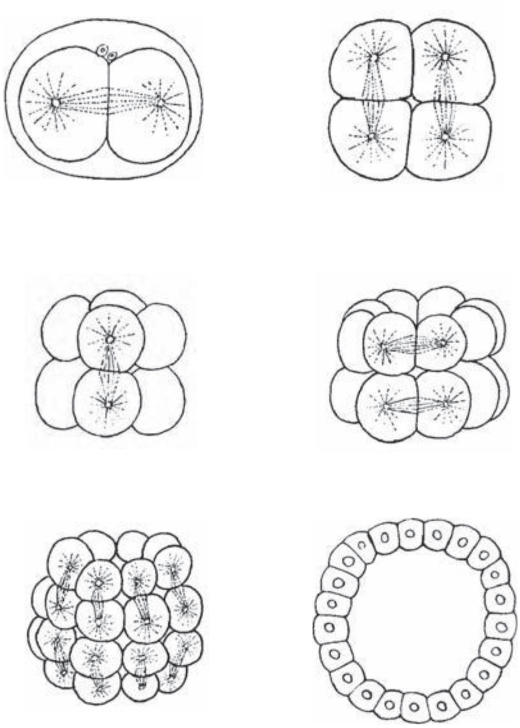

FIGURE 3.9. Typical holoblastic cleavage in a noninsect egg. At the lower right, the figure shows a saggital section. From Balinsky, B.I. 1970. *An introduction to embryology*, 3rd ed. Copyright W.B. Saunders Company.

prevents the first cleavage divisions from cutting through the entire egg, and their cleavage is more superficial, or **meroblastic**. Rather than dividing into the separate cells that would result from holoblastic cleavage, the nuclei divide without the formation of new cell membranes. Not all insect eggs are meroblastic; holoblastic cleavage takes place in collembolan eggs and those from some parasitic Hymenoptera that have little yolk.

Blastoderm Formation

During the meroblastic cleavage of most insects, the zygote nucleus undergoes mitotic division in the center of the egg cytoplasm, but the resulting daughter nuclei or **energids**, each surrounded by an island of cytoplasm, are not incorporated into new complete cells. Instead, after a series of mitoses, the energids migrate to the egg periphery with their islands of cytoplasm and continue to divide there (Figures 3.10A and 3.10B). The initial seven cleavage divisions are

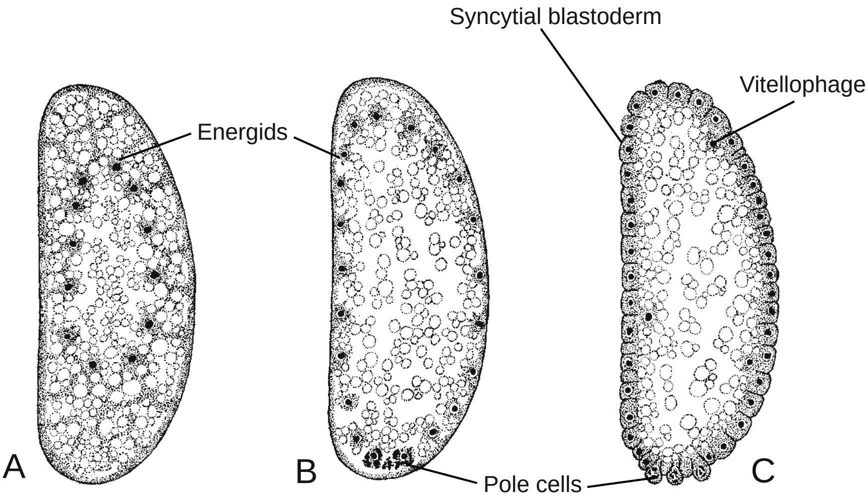

FIGURE 3.10. Migration of energids to the periplasm of an insect oocyte, forming the syncytial blastoderm. The vitellophages, agents of yolk digestion, and the pole cells, destined to form the germ cells, are derived from the energids. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

synchronous, but they become more asynchronous as they continue to occur in different regions of the egg; by the 10th division, they have reached the egg periphery. At the periphery, the energids first form a **syncytial blastoderm** that lacks any membranes, with all the cleavage nuclei contained within the common cytoplasm of the egg (Figure 3.10C). In *Drosophila*, the egg remains a syncytium for the first 2 hours of development, and the lack of any cell membranes undoubtedly facilitates the diffusion of morphogens. About two to three energids migrate to the posterior of the egg to form the **pole cells** that will give rise to the germ cells of the future adult, establishing the continuity of the germline early during embryogenesis. Their early removal from the normal course of cell divisions assures that their genetic integrity is maintained. The pole cells differentiate in the microenvironment present at the posterior pole largely because of the *oskar* gene product that appears to be necessary for their formation.

Other energids stop dividing mitotically, and rather than migrate to the periphery, about 20 remain in the yolk to form yolk cells, or **vitellophages**, which will be involved later in the digestion of yolk and the formation of the midgut epithelium. At about the time the pole cells have become distinct, membranes begin to develop around each of the nuclei of the syncytial blastoderm, now consisting of a population of about 5000, creating individual cells that form the **cellular blastoderm** (Figure 3.11). The cells of the blastoderm appear to be determined at this stage, because the experimental destruction of groups of cells at this time results in specific defects later in development.

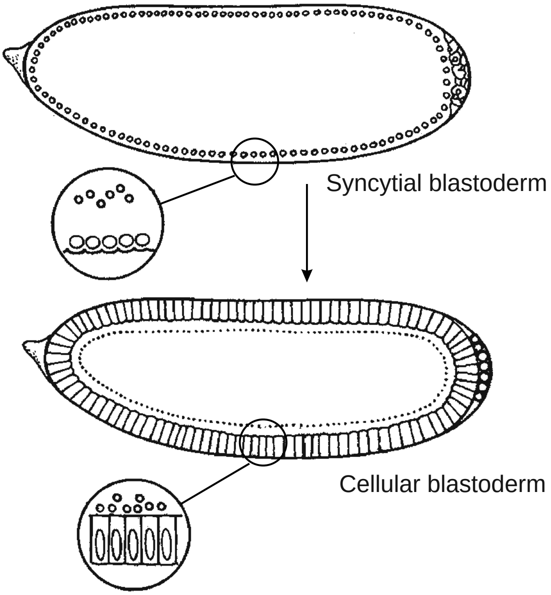

FIGURE 3.11. Formation of the cellular blastoderm from the syncytial blastoderm. From Lawrence, P.A. 1992. *The making of a fly*. Copyright Blackwell Scientific Publications. Reprinted with permission.

Formation of the Germ Band

As the division of the blastoderm continues, the rate of division changes in the ventral region, causing it to thicken as the cells become more columnar in shape. This thickened portion of the blastoderm becomes the **embryonic primordium** that will develop into the embryo, whereas the other cells of the blastoderm are designated as the **extra-embryonic ectoderm** (Figure 3.12). As the embryonic primordium increases in length, it forms the **germ band** that represents the ventral region of the future body. Continued proliferation of the germ band causes it to penetrate into the interior of the yolk mass, and the presence of morphogenetic gradients govern its continued determination and differentiation. As discussed previously, gradients of morphogens such as Bicoid protein at the anterior pole and the Nanos protein at the posterior pole activate or inactivate the transcription of specific genes that are associated with anterior or posterior structures.

The germ band is still a single layer of cells at this stage, but it takes an important step in development when it becomes a double layer by the process of **gastrulation**. In other animals, gastrulation involves the invagination of the ball of cells that has formed, but in insects, the invagination is hindered by the

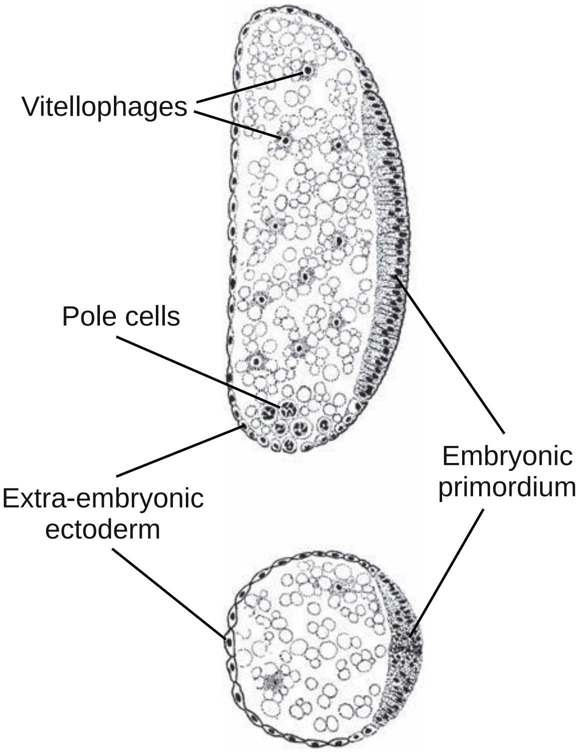

FIGURE 3.12. Development of the embryonic primordium and extra-embryonic ectoderm from the blastodermal cells. (Top) Saggital section. (Bottom) Cross section. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

large amount of yolk that is present. Instead, the formation of a multicellular layer occurs when some of the cells of the germ band migrate inward into the yolk (Figure 3.13). This reorganization produces a multilayered body consisting of different cell types that are now able to interact with each other in different ways. When the cells along the ventral midline of the germ band elongate and migrate upward, a longitudinal **ventral furrow** is created. Other cells of the germ band soon fill the ventral furrow, with this outer layer of cells remaining as the embryonic ectoderm while the invaginated cells proliferate to form the **mesoderm**. The migration of mesodermal cells is dependent on their expression of the *heartless* gene that encodes the *Drosophila* fibroblast growth factor receptor. With this initial expression, these cells acquire a different developmental fate and will form most of the internal organs. The two genes *twist* and *snail* are initially transcribed in a narrow band of cells destined to become mesodermal. *Twist* is a transcriptional activator for mesodermal genes and *snail* is one of its targets,

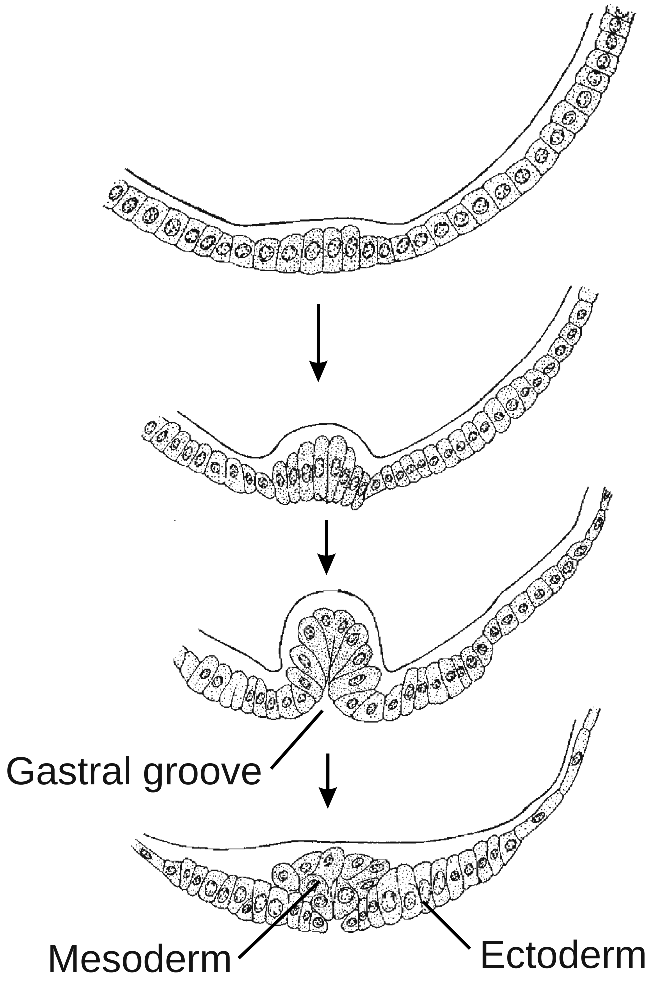

FIGURE 3.13. Formation of mesoderm from the migration of ectodermal cells. From Anderson (1972). Reprinted with permission.

and both genes are responsible for dividing the blastoderm into the ectodermal, endodermal, and mesodermal germ layers. The germ band extends with further growth and constitutes the trunk of the developing insect with the ectoderm on the outside and the mesoderm on the inside.

At this point, the products of the segmentation genes have divided the germ band of *Drosophila* into a series of 14 parasegments. Segmentation begins as the maternal effect genes activate or repress the gap genes to establish regions of segment identity. The concentrations of Bicoid, Hunchback, and Caudal proteins

determine the transcription patterns of the gap genes. The products of the gap genes then regulate the expression of the pair-rule genes, which when transcribed, further divide the broad gap gene regions into the parasegments. The parasegments partially correspond to the three mouthpart, three thoracic, and eight abdominal segments and will ultimately produce the anterior compartment of one segment and the posterior compartment of the next. The segment polarity genes assure that certain repeated structures appear in each segment, establishing the cell fates within each of the parasegments. Then, subsequent interactions of the gap and pair-rule genes regulate the homeotic genes that establish the identity of each segment and its characteristic structures. Homeotic mutants cause the misidentification of segments, often producing bizarre characters such as legs growing out of the head.

In all insects, a cascade of transcription factors is involved in the development of segmentation, but there are two ways of forming the segments. In the relatively advanced *Drosophila,* development is known as **long-germ band development** where the blastoderm corresponds to the entire portion of the embryo and all segments form at the same time. The cleavage nuclei do not migrate to the periphery simultaneously and reach some regions sooner than others. Long-germ band insects generally also have meroistic ovaries and nurse cells during oogenesis. All the parts of the embryo are determined before embryonic differentiation occurs. In contrast, many other insects engage in **short-germ band development**, where the blastoderm is short and corresponds to only anterior segments. Cleavage nuclei all migrate to the periphery simultaneously, but the posterior embryonic segments are added by growth once the blastoderm stage has been completed and gastrulation occurs. Embryos of short-germ band insects tend to engage in more physical movements within the egg during embryogenesis.

Blastokinesis and Dorsal Closure

With the subsequent formation of membranes, the germ band becomes separated from the extra-embryonic ectoderm. The germ band elongates and widens, carrying the margin of the extra-embryonic ectoderm with it. These **amniotic folds** extend to the ventral midline and merge to form a double layer of extra-embryonic cells that are ventral to the germ band surface. As the folds merge, a completely cellular membrane covers both the yolk mass and the germ band (Figures 3.14 and 3.15). The inner walls of the amniotic folds merge to form an internal cellular membrane, the **amnion**, continuous with the margin of the embryonic ectoderm. The amnion encloses a fluid-filled **amniotic cavity**. Once detached from the germ band, the extra-embryonic ectoderm is referred to as the **serosa**. The formation of the amnion separates the germ band from the extra-embryonic ectoderm and allows the germ band to engage in **blastokinesis**,

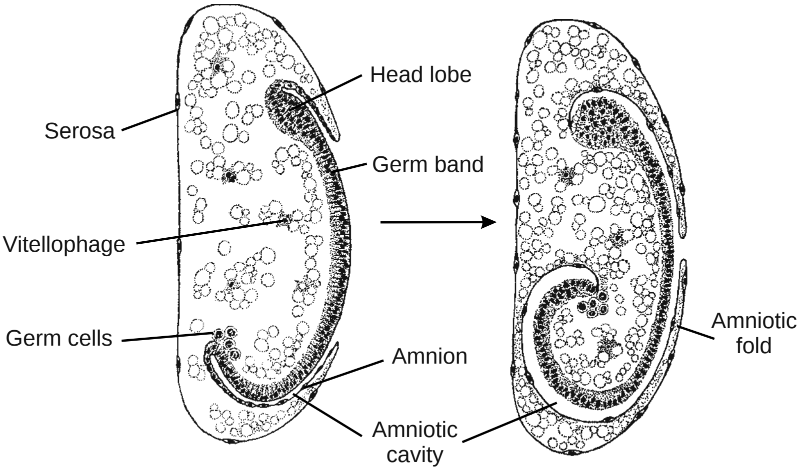

FIGURE 3.14. The formation of the amnion and the invagination of the germ band to free it from the serosa. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

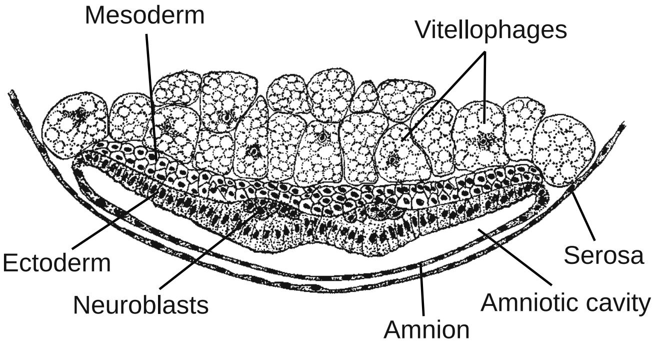

FIGURE 3.15. The formation of neuroblasts from ectodermal tissue and the proliferation of mesoderm. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

movements that take place within the yolk. These movements tend to be much more pronounced in hemimetabolous insects, which have a smaller germ band. The movements of the germ band reverse the relative positions of the yolk and the embryo. Earlier, the embryo was positioned within the yolk, but after blastokinesis and growth of the embryonic ectoderm over the dorsal portion to complete **dorsal closure**, the yolk is contained within the embryo (Figure 3.16).

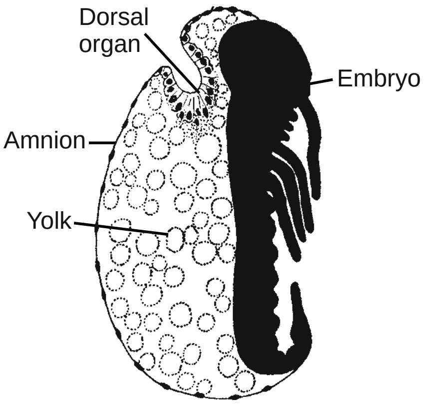

FIGURE 3.16. Dorsal closure of the embryo. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

Formation of the Gut

The development of various internal organs occurs after gastrulation and the generation of the new germ layers. Cells at the anterior and posterior ends of the embryo migrate inward to form the foregut, hindgut, and midgut. The foregut and hindgut arise from ectodermal cells, with some of the invaginating epithelia acquiring a new fate as the **endoderm** that will largely constitute the midgut. A reorganization of these endodermal cells forms sheets that enclose the yolk in a tube that creates the midgut epithelium. Vitellophages contained within the yolk that is enclosed by the midgut are integrated into the endoderm to form the definitive midgut epithelium (Figure 3.17). Thus, the midgut is formed by the tips of the stomodeum and proctodeum, as well as from those energids that became vitellophages. The local expression of several transcription factors, including those encoded by the genes *Sex combs reduced*, *extradenticle*, and *homothorax*, determine where in the gut the salivary glands will form from primordial cells. The visceral mesoderm arises from a small group of cells in each segment and forms bands on each side of the embryo that serve as pathways for endodermal migration. This cell migration results in the further midgut differentiation into caecae and the proventriculus, dependent on the expression of four homeotic genes: *Sex combs reduced*, *Ultrabithorax*, *Antennapedia*, and *abdominal-A*. The movement of cells to form foregut structures is dependent on the spatial expression of **integrins**, cell surface adhesion receptors that mediate cell-cell interactions. Shortly before hatching occurs, the blind ends of the foregut and hindgut break down and the continuity of the digestive tract is established.

The elongating hindgut becomes subdivided into the three regions of the small intestine, large intestine, and rectum, each of which contains cells of

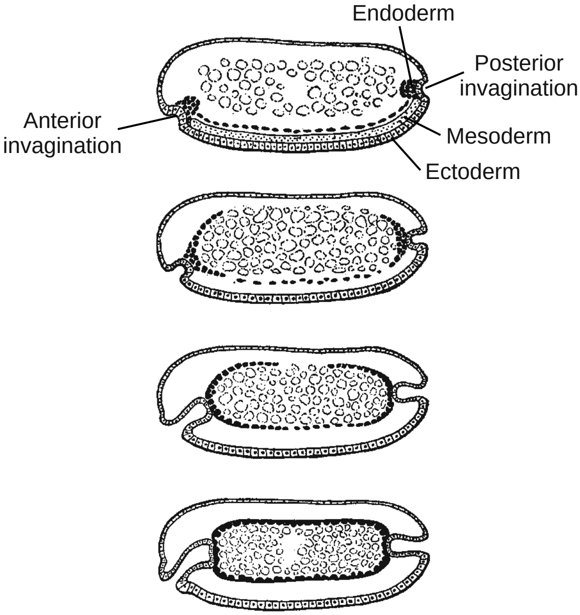

FIGURE 3.17. Formation of the foregut and hindgut from ectodermal invaginations and the development of endoderm that forms the midgut. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

differing size. The cells of the large intestine undergo continued DNA replication to become significantly larger than the others. Several genes, including *drumstick bowl* and *lines*, encode transcription factors that regulate this hindgut patterning and cell rearrangement. The proctodeal invagination also gives rise to the Malpighian tubules at its juncture with the posterior midgut. Primordial cells located between the midgut and hindgut that express the transcription factors Krüppel and Cut form buds, and cells from the proctodeum that move into the buds express the *walrus* gene and Krüppel. Subsequent cell movements to form the mature Malpighian tubule require the *hedgehog* and *wingless* signaling pathways.

Formation of the Nervous System

The nervous system arises from ectodermal cells in the ventral region of the germ band. Proliferation of the neuroblasts in portions of the embryonic ectoderm of the germ band create longitudinal thickenings on either side of the ventral midline, producing a **neural groove** and **neural ridges** (Figure 3.18). The neural ridges result from the proliferation of **neuroblasts** that differentiate from the ectoderm and give rise to the nervous system. Neuroblasts are present

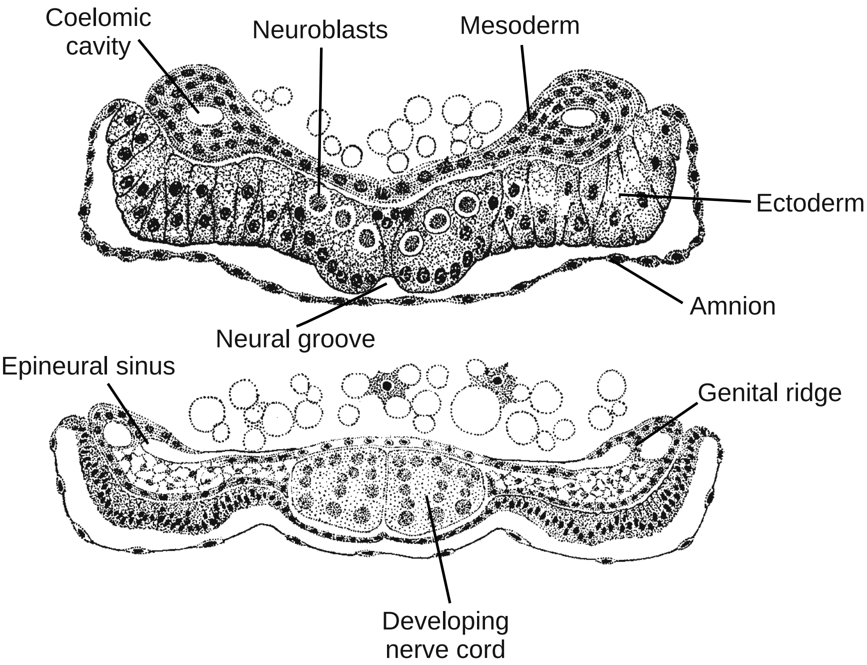

FIGURE 3.18. Formation of the ventral nerve cord from neuroblasts. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

initially as scattered cells that are delaminated from the neurectodermal layer. There are about 80 neuroblasts in *Drosophila* and 130 in the grasshopper. The commitment of the ectodermal cells to become neuroblasts rather than epidermal cells results from the expression of a group of **proneural genes**, including *achaete*, *scute*, and *lethal of scute*. The subsequent expression of **neurogenic genes** inhibits any neighboring cells from becoming neuroblasts. Three groups of neuroblasts that proliferate in the anterior region ultimately produce the protocerebrum, deutocerebrum, and tritocerebrum of the brain. Those in other segments give rise to the subesophageal and abdominal ganglia. These proliferative clusters of neuroblasts are surrounded and ultimately wrapped by glial cells. The growing axons and dendrites in the embryo bear a growth cone that is sensitive to external guidance cues, and neuron connectivity is choreographed by various signal molecules that bind to receptors on the cone. Neurons are initially attracted to the midline of the embryo, but once they reach it they are repelled and move away. The *roundabout* (*robo*) gene encodes an axon guidance receptor whose ligand is the **Slit protein**, produced at the *Drosophila* midline. Slit proteins are also expressed by ventral midline cells in vertebrates. Proteins called **netrins** initially attract the axons to the midline but they are later repelled by the Slit

produced there when the axons express the Robo protein. When Slit repels cells expressing Robo, it causes the axons to continue traveling past the midline.

Formation of Eyes

The compound eye of *Drosophila* develops from the eye-antenna imaginal disc, an early invagination of the ectoderm. Specification of the compound eye from these cells results from the expression of several transcription factors, including the products of the *eyeless* and *twin of eyeless* genes, both of which are homologs of the *Pax-6* gene that is involved in the development of the vertebrate central nervous system and eye. Primitive insects have only one homolog while more advanced insects have both. *Pax-6* appears to be a master control gene for eye development in both vertebrates and invertebrates and has also been found in planaria and nematodes. A truly eye-opening experiment showed that the ectopic expression of the Pax-6 protein from the mouse in a *Drosophila* host induced ectopic compound eyes on the legs, antennae, and even wings of the flies. The most intriguing implication of this developmental relationship is that the eyes of both vertebrates and arthropods may ultimately be homologous, with the generation of a prototype eye by *Pax-6* and divergent evolution subsequently producing the overall differences in origin and structure of the various eye types.

Formation of Other Internal Organ Systems

The tracheal system originates from 10 clusters of ectodermal cells on either side of the *Drosophila* embryo, with each cluster consisting of about 90 cells that invaginate to form sacs under the direction of the *trachealess* gene that encodes a transcription factor expressed in all the tracheal cells during their development. The number of cells that give rise to the tracheal system is fixed at this point, and further morphogenesis occurs only by cell migration without cell division. These cells migrate to form six branches followed by about 20 secondary branches and hundreds of tertiary branches that grow and fuse to form the interconnected tracheal network. There are four distinct types of tracheal tubes that build the tracheal system, whose fates are assigned after the establishment of the identities of the ectoderm as anterior-posterior and dorso-ventral regions.

Branching is guided by the nontracheal cells surrounding these invaginating tracheal cells that express the fibroblast growth factor-like ligand encoded by the *branchless* gene. The Branchless (Bnl) ligand is expressed in clusters of cells arranged around the tracheal sacs and binds to the Breathless (Btl) receptor expressed by tracheal cells to mediate their branching and migration. As the primary branches grow toward the cluster of cells expressing *branchless*, the gene turns off and the branch may continue to grow toward another *branchless*-

expressing cluster. Areas of secondary branching are associated with the additional expression of the *pointed* gene and terminal branching with *blistered* expression. The Bnl/Btl signaling pathway thus establishes the overall branching pattern, with the branching migration and subsequent guidance directed by additional gene expression, the Slit protein, and by the local demand for oxygen in the target cells.

Mesodermal tissue gives rise to most of the other internal organs of the insect (Figure 3.19). The cells of the germ band give rise to two cell layers with two mesodermal strands running the length of the body that are formed during gastrulation and dependent on the fibroblast growth factor receptor for migration. Later in development, a pair of coelomic cavities develops in each segment, forming a tube through the thoracic and abdominal segments. The mesoderm is differentiated into two layers, the inner **splanchic mesoderm** that forms the visceral muscles and the **somatic mesoderm** that gives rise to the skeletal muscles. These cell types differentiate based on their expression of the *twist* gene encoding the Twist transcription factor, with those expressing a high Twist concentration becoming skeletal muscles of the somatic mesoderm and those expressing low Twist concentrations becoming visceral and heart muscles of the splanchic mesoderm. The subdivision of this mesodermal tissue is regulated by the segment polarity genes *engrailed*, *hedgehog*, and *wingless*. The muscle precursor cells fuse with other mesodermal cells from the surrounding area and continue their muscle-specific differentiation program. The fat body is derived from the somatic layer that is not in contact with the ectodermal layer. At the junction

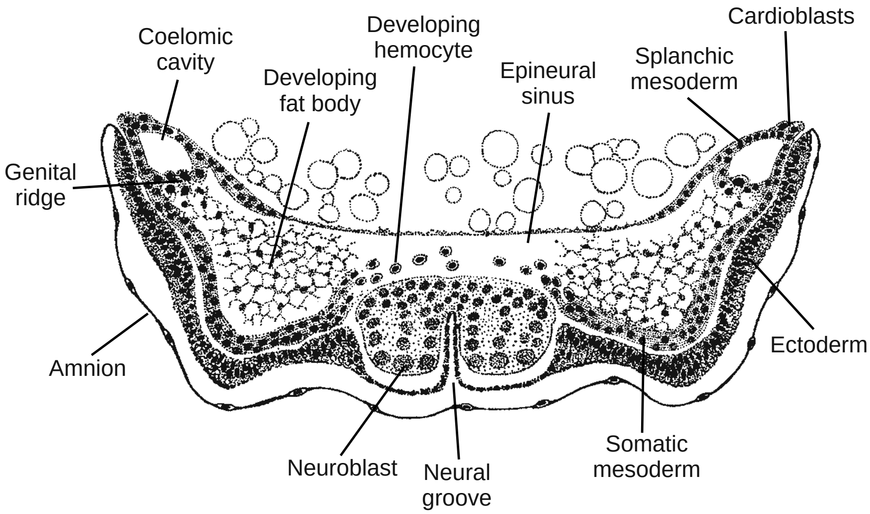

FIGURE 3.19. Mesodermal tissues that give rise to the internal organs. From Johansen, O., and F. Butt. 1941. *Embryology of insects and myriapods*. Copyright McGraw-Hill Education. Reprinted with permission.

of the splanchic and somatic layers, cardioblasts differentiate that ultimately give rise to the heart. Mesodermal tissue also forms a **genital ridge** that encloses the germ cells and forms the reproductive organs. When the median strand of mesoderm breaks down, some of its cells become hemocytes. Mesodermal somites acquire a lumen in each lateral half that eventually becomes the body cavity, or hemocele. The **epineural sinus** in which the hemocytes lie merges with the lumen to become the hemocele.

The reproductive system is constructed around the **pole cells** that had differentiated early during embryogenesis. The pole cells become the germ cells of the future adult, with surrounding mesodermal tissue enclosing those cells to form the germaria of the ovarioles and the follicles of the testes. Mesoderm also gives rise to the lateral oviducts and vasa deferentia. Invaginations of the ectoderm form the median oviduct and ejaculatory duct.

A summary of the contributions of all these cell lineages toward adult structures is shown in Figure 3.20.

Eyespot Pattern Formation

The species specific color pattern on the wings of lepidopterans results from the spatial ordering of pigmented scales that are produced by the underlying epidermal cells just before adult eclosion. The patterns provide the bearers with aposematic or cryptic coloration that may be used for defense or to identify mates.

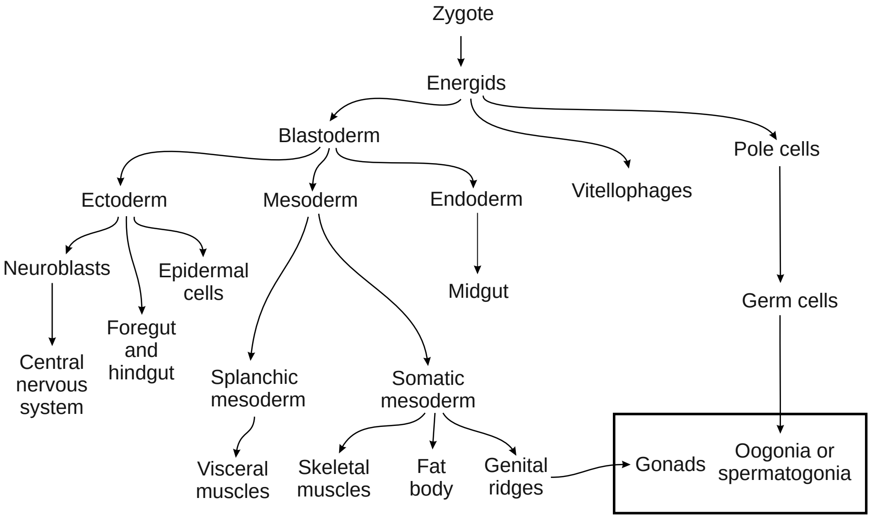

FIGURE 3.20. Cell lineages and derivation of tissues in the mature insect. Adapted from Chapman 1998. *The Insects: Structure and Function*. Cambridge University Press.

The epidermal cells each bear a single scale into which is deposited a single pigment and when combined with neighboring scales produce the larger patterns of bands and eyespots. Eyespots consist of concentric rings of different colors and provide protection by startling a potential predator or fooling it into thinking its prey is larger than it actually is. Even genetically identical individuals can display different patterns depending on the environment they are exposed to during development. Some butterflies display a seasonal polyphenism of alternative phenotypes that are adapted for the particular season in which the adults spend their lives.

The wings of lepidopterans originate as paired imaginal discs that begin development during the last larval instar and evert at metamorphosis to form pupal wings. It is during this developmental period that pattern development takes place. Eyespot development occurs around a small group of epidermal cells called the **focus** that acts as a developmental organizer to induce the surrounding cells to activate the enzymes that synthesize certain pigments. Damage to the focus during the pupal stage results in reductions in the size of the eyespot of the adult wing, and transplantation of the focus to another area of the pupal wing moves the eyespot that develops in the adult. The model for eyespot formation postulates that a cone-shaped gradient of a morphogen diffuses across the surrounding field of cells, and these cells interpret the gradient based on its concentration and synthesize pigment accordingly (Figure 3.21). During the last larval instar,

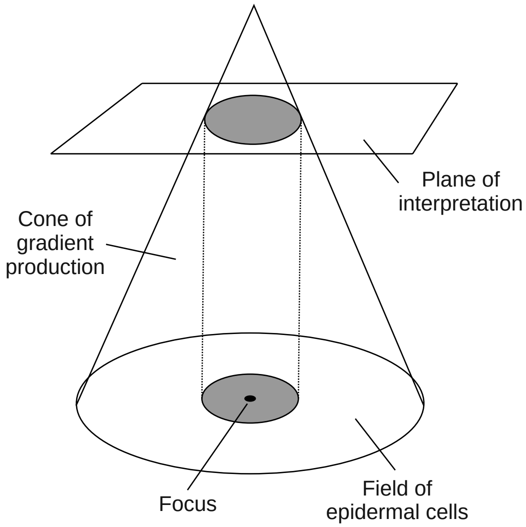

FIGURE 3.21. A gradient of a morphogen produced by a small group of epidermal cells that form a focus on the wings of lepidopterans. The concentration of the gradient is represented by a cone that is projected on a plane of epidermal cells that interpret the gradient according to their genetic instructions.

the expression of the *Distal-less* (*Dll*) gene encodes a transcription factor that initiates pattern in wing subdivisions and later in epidermal cells, moving inward from the wing edge. Ultimately, *Dll* is expressed in those cells that will form the focus. Other genes including *hedgehog*, *patched*, and *Cubitus interruptus* are also expressed specifically in the focus and the areas surrounding it, but the interactions of the entire morphogenetic complex have yet to be identified. Although the synthesis of pigment in epidermal cells is dependent on the signaling activity from the focus, these other transcription factors may regulate the thresholds at which the cells respond to the primary signal. The focus is not alone in specifying pattern; wing veins not only act as compartment borders but may also be inductive sources of pattern. There is some evidence for the involvement of ecdysone signaling in color pattern development, as the timing of ecdysone release after the larval-pupal molt can produce different patterns, as in seasonal morphs.

Endocrinology of Embryonic Development

It has been much more difficult to characterize the role of hormones during embryogenesis than during postembryonic development. Hormonal functions in the larval and adult stage are normally demonstrated by gland extirpation and hormone replacement therapy, procedures that are not possible for the egg. Consequently, endocrinology of the embryo has largely involved the isolation and identification of the hormones and their correlation with significant events during embryogenesis. The sources of these hormones and their fluctuations during embryogenesis were at first somewhat of a puzzle, because in the absence of embryonic glands there was no obvious place they could be synthesized. Their presence well before the embryonic endocrine organs have been formed is a result of their maternal synthesis during vitellogenesis. They are packaged into the egg largely as inactive hormone conjugates and released from the conjugates as active hormones when they are required.

The origin of the ecdysteroids in the adult female destined to be packaged in the egg was also somewhat puzzling, because the source of ecdysteroids for larval molting, the prothoracic glands, degenerate shortly after the molt to the adult, and no prothoracic glands are present in the female to produce the hormone during vitellogenesis. However, an important discovery was that the follicle cells in the developing ovaries of many adult insects could also synthesize ecdysteroids, and this finally demonstrated how the hormones were produced to be incorporated into the egg.

The fluctuations of embryonic ecdysteroid titers appear to occur when the hormones are released from their storage as inactive conjugates. Only later, when embryonic endocrine organs are formed, are the embryos capable of synthesizing new hormones. A large number of ecdysteroids have been isolated from

insect eggs, primarily ecdysone, 20-hydroxyecdysone, 26-hydroxyecdysone, 2-deoxyecdysone, and 20,26-dihydroxyecdysone. Several metabolic intermediates between cholesterol and ecdysone have also been isolated. The maternal conjugates are often bound to vitellin and stored in yolk granules. The 20-hydroxyecdysone that appears in nondiapausing *Bombyx mori* eggs originates from both the conversion of maternally derived ecdysteroid conjugates and embryonic synthesis. The ecdysteroids are not present, however, in diapausing eggs, and their deficiency may bring about the embryonic diapause.

Molting also occurs during embryonic development in many insects, just as it does during postembryonic life, and often before the prothoracic glands are functional. The embryo of *Locusta migratoria* produces four distinct cuticles, including the serosal cuticle and three embryonic cuticles, the last of which is the cuticle of the first instar larva. Associated with these molts are ecdysteroid peaks, and the ecdysteroids are packaged as conjugates of phosphate or yolk proteins, with the increases in the titers of active hormone resulting from their release of the conjugates. The enzyme ecdysteroid-phosphate phosphatase (EPPase), which resides in the yolk and amnioserosa, catalyzes this conversion of ecdysteroid conjugates to free ecdysteroids. The levels of ecdysteroids typically peak with the deposition of these cuticles, and the coincidence of ecdysteroid peaks with these molts strongly suggests their involvement in embryonic molting. The embryonic prothoracic glands are formed after blastokinesis and may then begin to synthesize ecdysteroids. Ecdysone signaling also controls the morphogenetic movements of the embryo. Ametabolous and hemimetabolous insects have a **pronymphal** stage that precedes the first larval instar, thought to be a continuation of embryological development after hatching occurs. While enclosed in the embryonic cuticle, a peak of ecdysteroid causes the molt to the pronymphal stage, with another ecdysteroid peak signaling the molt to the first stage larva.

Little is known about the contributions of JH during embryogenesis. At least five different juvenile hormones have been isolated from insect eggs, and topical treatment with JH or its analogs often affects embryonic development. There are correlations between the titers of JH and significant events during embryogenesis, with increased levels reported before and during blastokinesis and at the end of embryonic development.

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CHAPTER 4

Reproductive Systems

Insects are the preeminent animals on our planet. Whereas other physiological systems provide the potential for their success, their reproductive systems enable that potential to be realized. Although short-lived insects have a relatively brief reproductive opportunity, they compensate with a diverse assemblage of reproductive strategies that allow prodigious numbers of offspring to be produced under a wide range of ecological circumstances. These strategies include parthenogenesis, paedogenesis, polyembryony, functional hermaphroditism, viviparity, and the more common bisexual reproduction and oviparity.

The most impressive reproductive outputs are found in the social insects that have castes specializing in egg production. One queen honey bee, living for up to 5 years, can produce up to 600,000 eggs during her first 3 years of life. Queen termites lay about 10 million eggs each year over their 15- to 20-year life spans. Consider the reproductive potential of the nonsocial female mosquito, *Aedes aegypti*. She can produce about 125 eggs from each blood meal she ingests, and those eggs can develop to adults in about 10 days after she feeds. If one female mosquito began to reproduce in the spring with a single blood meal and all her offspring survived and likewise reproduced, by the end of the summer there would be more than 7×10^{21} mosquitoes. Their biomass at the end of these three months would be more than 1×10^{19} g, about 33,000 times the weight of

the total human population. How many other animals weighing in at 2 mg can boast of such productivity? Of course, insect populations never reach those tremendous numbers because of the high mortality from the many predators that depend on insects as food. A system with an extremely high reproductive potential is the price they must pay for occupying their particular ecological niches, and the design of their reproductive systems is the result of selective pressures encountered in the process of meeting this potential.

The organizations of both male and female reproductive systems are fairly similar. The germ cells of the reproductive organs originate from the **pole cells** that are among the first to differentiate during embryogenesis. Together with mesodermal tissue, they form the reproductive organs of the adult. A pair of gonads is connected by individual ducts to a common duct, constructed from both mesodermal and ectodermal origins. Both reproductive systems are coordinated by hormones and transcription factors, which are ultimately regulated by physiological and environmental factors.

FEMALE REPRODUCTIVE SYSTEMS

The reproductive system of female insects consists of a pair of **ovaries** that are connected to a **median oviduct** by a pair of **lateral oviducts** (Figure 4.1).

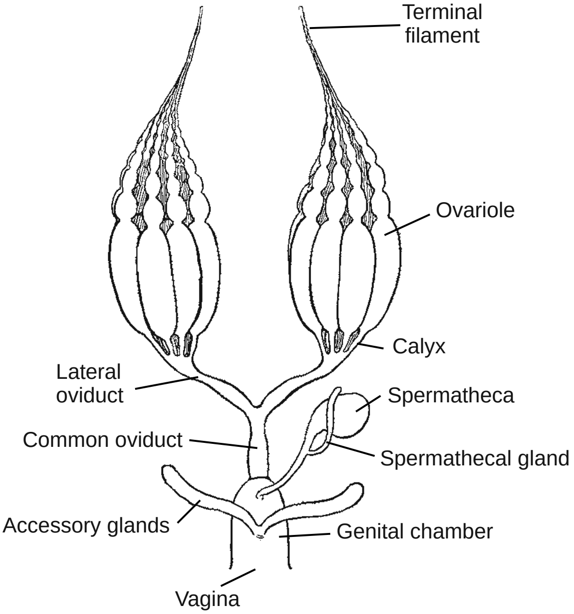

FIGURE 4.1. The generalized female reproductive system. From R.E. Snodgrass (1935). *The Principles of Insect Morphology*. Copyright by Ellen Burden and Ruth Roach. Reprinted with permission.

During embryogenesis, most of the ovary develops from the splanchic mesoderm. Those portions bearing the germ cells develop into the ovary, and other anterior cells give rise to a **suspensatory ligament** at the anterior end that unites all ovarioles and anchors the ovary to the body wall. The lateral oviducts connect to the ovaries at their posterior ends and combine into the ectodermally derived common oviduct.

The **ovarioles** are a series of tapering egg tubes, the functional units of the ovary that contain a progression of developing **oocytes** (Figure 4.2). Oocytes develop and grow sequentially within the ovarioles in an assembly line fashion. The number of ovarioles in each ovary varies tremendously depending on the size and reproductive strategies of the particular insect species and largely determines fecundity. At one extreme, there are apterygotes with only one ovariole per ovary. At the other extreme are the queens of some social insects that may have more than 1000 per ovary. The queen termite, *Eutermes*, has more than 2000. Although ovariole number is genetically determined, it results from alterations in cell differentiation during the later stages of larval development and can be modified by the diet of the immature stages. The number of ovarioles

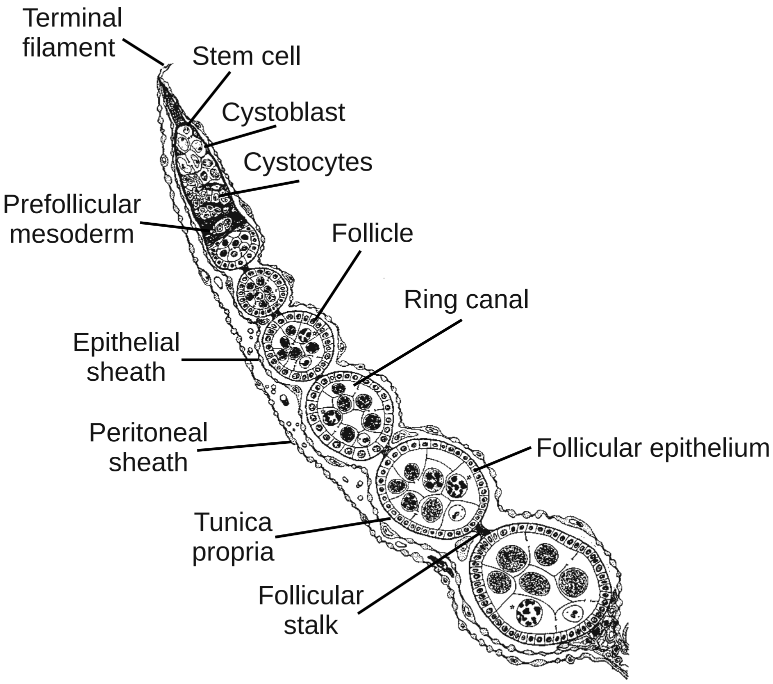

FIGURE 4.2. A chain of oocytes within an ovariole. Oocytes arise from the stem cells in the germarium and descend into the vitellarium surrounded by follicle cells. The follicle cells produce the chorion around the oocyte and then degenerate. From Koch et al. (1967). Reprinted with permission.

per ovary in blow flies can vary from 51 to 165 depending on nutrition. The most common range of ovariole number in most insects is from 4 to 10 per ovary.

Events in the Germarium

Drosophila oocytes begin their growth at the anterior end of the ovariole just beneath the **terminal filament** where the ovarioles are clustered. Here, the **germarium** contains the **germline stem cells** within a special microenvironment, the **stem cell niche**. Within this niche, the stem cells are surrounded by three differentiated somatic follicle stem cells, the **terminal filament cells**, **cap cells**, and **inner sheath cells** (Figure 4.3). Stem cells contain a cytoplasmic

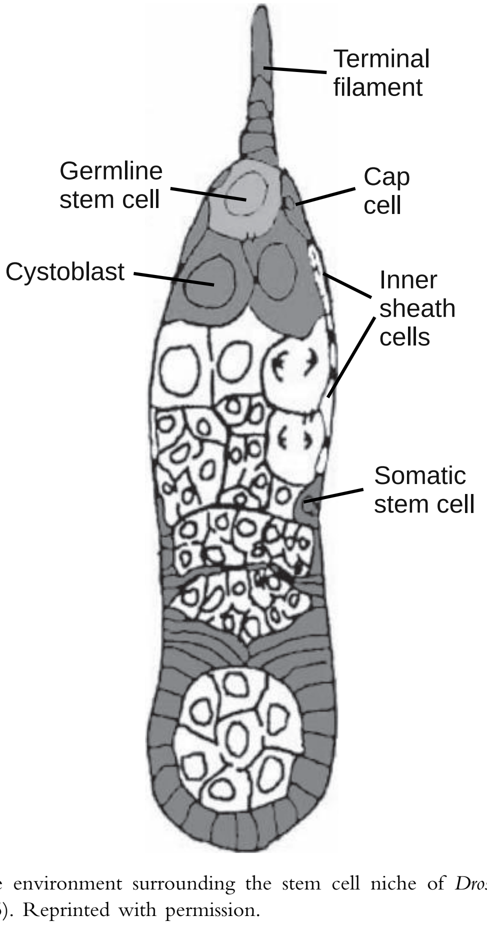

FIGURE 4.3. The environment surrounding the stem cell niche of *Drosophila* females. From Yamashita et al. (2005). Reprinted with permission.

organelle called a **spectrosome**, comprised of the cytoskeletal proteins α- and β-spectrin and ankyrin. As the mitotic divisions continue, the spectrosome becomes branched and gives rise to the **fusome** of daughter cells that is responsible for anchoring the mitotic spindle during cell division (Figure 4.4).

When the germline stem cell divides asymmetrically, the daughter cell closest to the terminal filament and cap cells remains a stem cell, but the one nearer to the inner sheath cells differentiates into a **cystoblast**. Intercellular signals within the niche coordinate the populations of both the germline stem cells and somatic stem cells to maintain their numbers. Insulin-like peptides regulate the rate at which germline stem cells divide in *Drosophila*, coordinating the response of reproductive tissues to nutritional state. Terminal filament cells and cap cells express several signaling molecules that maintain both germ cells and stem cells within the niche. The gene *decapentaplegic* (*dpp*) is active in the niche and its mRNA is expressed in cap cells. Its expression represses the expression of *bag of marbles* (*bam*), which is necessary for cystoblast differentiation and thus maintains the germline stem cells in the niche. The genes *piwi* and *Yb* similarly maintain the germline stem cells and prevent their depletion along with two RNA binding proteins, Nanos (nos) and Pumilio (Pum). Hedgehog (Hh) and wingless (Wg) proteins are also expressed by terminal filament and cap cells and maintain the somatic stem cells. The somatic stem cells produce the follicle cells that surround the newly formed cystoblast as it undergoes cell division with incomplete cytokinesis, which in *Drosophila* ultimately gives rise to a cyst of 16 cystocytes that are connected to each other by a series of cytoplasmic bridges, or **ring canals** (Figure 4.5). The fusome extends through each of the ring canals as the cyst enlarges and is asymmetrically distributed to daughter cells.

As the cystocyte complex in *Drosophila* moves down the ovariole, it enters the region of the ovariole called the **vitellarium** once it has been completely surrounded by follicle cells. The follicle cells provide both structural and functional roles; they surround the cystocyte complex and provide the cell signaling interactions that later establish the polarity of the developing oocyte. In higher dipterans, the follicle cells also express the vitellogenin gene. One of the two cystocytes that contains four ring canals and has inherited more fusome than the other will become the oocyte and subsequently undergo meiosis (Figure 4.4). The ring canals are the conduits for a number of proteins and mRNA that polarize the oocyte and provide a pattern for development to occur. The remainder of the cystocytes becomes nurse cells.

Other Structures in the Female Reproductive System

At the posterior end of the ovariole, the **calyx** joins it to the **lateral oviduct**. The **common oviduct** is ectodermal in origin and is lined with cuticle. The lateral oviducts are generally considered to be mesodermal along with the follicle

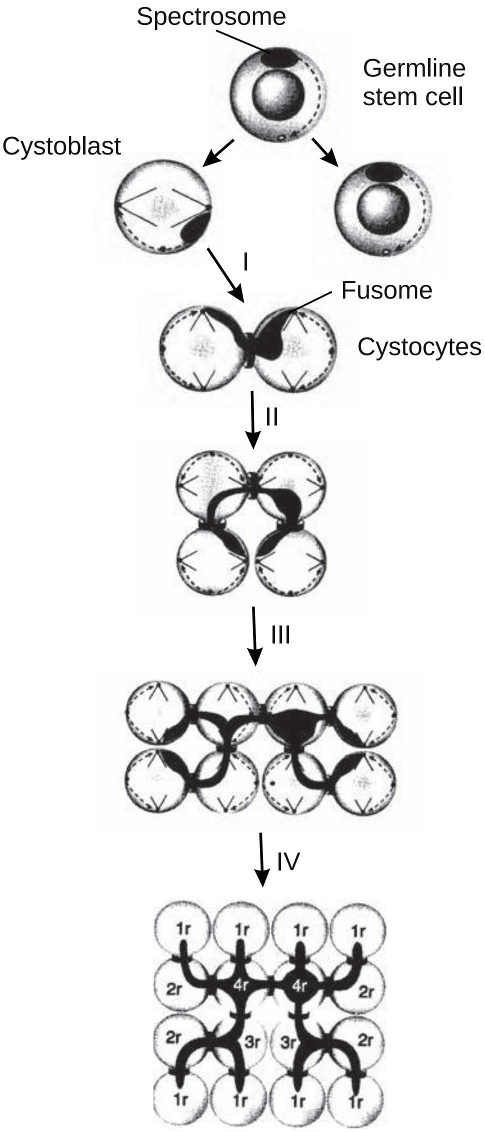

FIGURE 4.4. The division of germline stem cells and the formation of cystocytes connected by ring canals. The fusome divides asymmetrically, and one of the two cystocytes with four ring canals and the greatest amount of the fusome will become the oocyte. From Dansereau et al. (2005). Reprinted with permission.

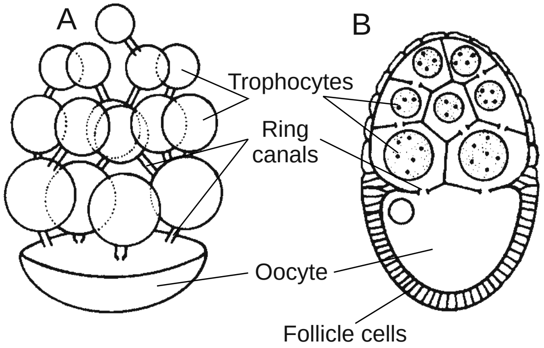

FIGURE 4.5. The differentiation of cystocytes into trophocytes and an oocyte, still connected by ring canals, in 3 dimensional (A) and saggital (B) views. From De Loof. (1983). Reprinted with permission.

cells and ovariole wall. A thin acellular membrane, the **tunica propria**, covers the ovariole from the terminal filament to the calyx (Figure 4.2).

Specialized structures are associated with the common oviduct. The **female accessory glands**, also known as **collaterial glands**, are modified dermal glands. These glands produce cement that allows the deposited eggs to be attached to the substrate or glued together. In some insects that retain their eggs after they hatch, such as the tsetse, *Glossina*, the accessory glands produce a nutritive secretion that nourishes the larvae during their entire larval period. In cockroaches and mantids, the female accessory glands produce the hardened egg case, or **ootheca**. Oothecae are comprised of cross-linked protein only, without the chitin that stabilizes the cuticle. The synthesis of the cockroach ootheca is described in Chapter 2.

The fertilization of insect eggs generally takes place well after insemination occurs, and the **spermathecae** store the sperm until they are required. Also ectodermal in origin, the spermathecae open into the common oviduct and release sperm as the fully formed eggs pass by. They are often present as multiple storage organs that better allow females to segregate and manipulate the sperm. In some *Photinus* fireflies, sperm that are stored in a secondary spermathecae show a higher degree of viability that those stored in the primary spermathecae, and they may provide the female with a mechanism to segregate sperm and participate in sperm competition when multiple mating occurs. The spermathecal duct may contain glycogen deposits that can serve as an energy source for the sperm as they pass through to the egg. Relatively long-lived female social insects must store and maintain sperm for years, during which time the sperm are actively respiring and subject to damage from reactive oxygen molecules. High levels of antioxidative enzymes are produced by mated honey bee queens and are present in their spermathecae, suggesting that they may protect the sperm from oxidative damage. In the Therevidae, a group of brachycerous Diptera, an

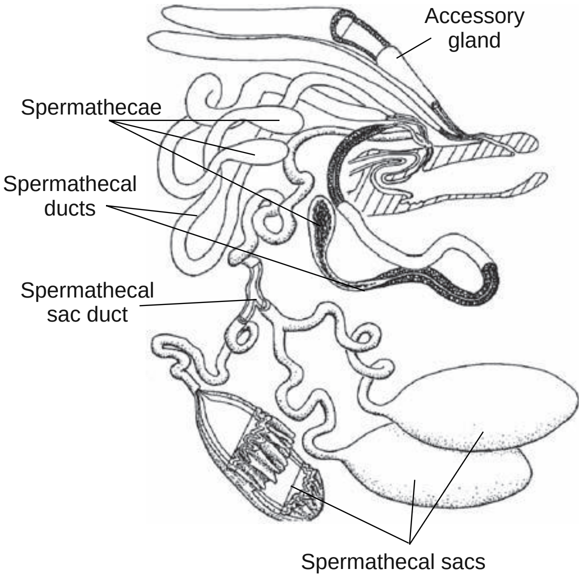

FIGURE 4.6. The spermathecal sacs in the female reproductive tract of therevid dipterans. From Winterton et al. (1999). *International Journal of Insect Morphology and Embryology* 28: 273–279. Copyright Elsevier. Reprinted with permission.

additional spermathecal sac joins the bursa copulatrix through a duct (Figure 4.6). Its function may be absorptive and able to accommodate sperm and male accessory gland material prior to their transfer to the spermathecae.

Nearest its opening to the outside, the common oviduct may be modified into a **genital chamber** that is capable of incubating eggs internally. The **bursa copulatrix** is an additional pouch within the chamber into which sperm are first deposited after mating. The sperm leave the bursa and then move into the spermatheca where they are stored more permanently. In more primitive insects, the bursa may contain a series of toothlike structures that disrupt the spermatophore in which the sperm are contained and facilitate their release. It may also secrete chemical signals into the hemolymph when it is filled with sperm to signal to the female that mating has occurred. In some tephritid flies, an additional sperm storage organ, the **fertilization chamber**, is present as a cuticular extension of the ventral wall of the bursa copulatrix (Figure 4.7). The chamber is initially filled with sperm, but these are then transferred to the spermathecae for long-term storage with a smaller number remaining in the chamber that are used for the fertilization of eggs. Sperm are shuttled back to the fertilization chamber from the spermathecae to maintain a small number there. The chamber appears to operate as a staging area for sperm that are used for immediate fertilization instead of releasing, and potentially wasting, larger numbers from the spermathecae when an egg is ovulated.

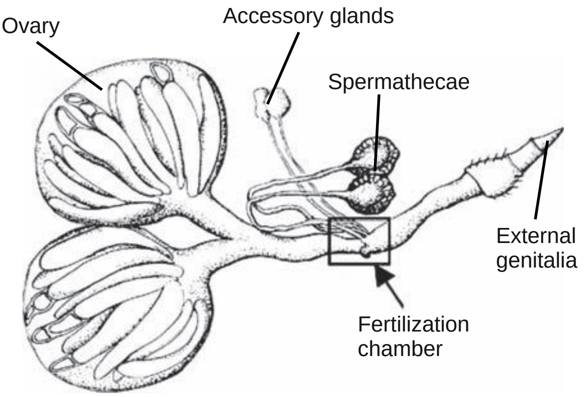

FIGURE 4.7. The fertilization chamber in the female reproductive tract of tephritid dipterans. From Twig and Yuval (2005). *Journal of Insect Physiology* 51: 67–74. Copyright Elsevier. Reprinted with permission.

Types of Ovarioles

The ovariole is the basic unit of egg production and can be classified into two major types that differ in the way that RNA and other nutrients are supplied to the oocyte. In the **panoistic** type, the germarium contains only oogonia, primary oocytes, and mesodermal prefollicular tissue (Figure 4.8A). The follicular epithelium supplies the oocyte with most of its nourishment, with the selective amplification of the genes for ribosomal RNA in the oocyte nucleus to supply the large number of ribosomes that are necessary for protein synthesis during development. The ring canals that serve as bridges between germline cells are absent. Panoistic ovarioles are believed to be the most primitive of the two types, found in the more primitive insect orders, including the Archeognatha, Thysanura, Odonata, Orthoptera, Plecoptera, and Isoptera.

A more effective way to provide oocytes with the materials they need for growth is to use sister cells whose genomes can be increased by endomitosis. In the more advanced **meroistic** ovariole, stem cells, primary oocytes, prefollicular tissue, and **nurse cells**, or **trophocytes**, are contained within the germarium. Meroistic ovarioles can be further divided into **telotrophic meroistic** and **polytrophic meroistic** (Figure 4.8B and C). In both types of ovarioles, the trophocytes provide the oocyte with RNA, proteins, and ribosomes through much of their development that are otherwise provided only by the oocyte itself in panoistic ovarioles. In the telotrophic meroistic ovariole, the nurse cells remain in the apex of the ovariole and feed the oocyte through a nutritive cord as it descends alone. The difference in electrical potential between the trophocytes and the oocyte may be responsible for the flow of materials into the cytoplasm of the oocyte. Toward the end of oocyte maturation, the cytoplasmic cord is

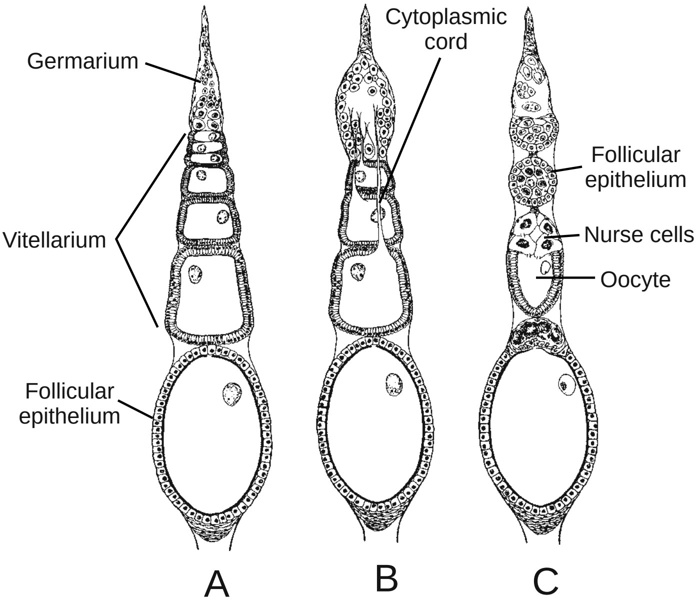

FIGURE 4.8. The three major types of insect ovarioles. A. The panoisitic ovariole, where nourishment for the oocyte comes only from the follicular epithelium. B. The telotrophic meroistic ovariole, with a cytoplasmic cord that connects the nurse cells that remain in the germarium with the descending oocyte. C. The polytrophic meroistic ovariole with the nurse cells contained within the follicle. From Schwalm (1988). Reprinted with permission.

broken. Telotrophic meroistic ovarioles are found in most Ephemeroptera, Hemiptera, and some Coleoptera.

Most holometabolous orders have a polytrophic meroistic ovariole, in which the oocyte is one of a group of interconnected cells, or **cystocytes**, that all descend together within the follicle (Figure 4.8C). The nurse cells are located at the anterior of the follicle and the oocyte at its posterior end, all surrounded by the follicular epithelium. The nurse cells are sister cells of the oocyte that arise by mitotic divisions and remain connected to the oocyte and each other by the cytoplasmic bridges called ring canals, forming a cystocyte complex (Figure 4.4). The number of nurse cells per oocyte is $2^n - 1$, where n is the number of species-specific mitotic divisions that occur during development. The nurse cells also undergo endomitotic chromosome replications and become polyploid, enhancing their ability to produce ribosomal RNA. This differs from panoistic ovarioles where the oocyte selectively amplifies its own rRNA. When their mitotic divisions are complete, oocyte-specific factors begin to move by polarized transport to the one cystocyte that will ultimately differentiate into the oocyte. In *Drosophila*, the oocyte always arises from one of the two oldest cystocytes that have the largest number of ring canals. When the nurse cells have

passed all their contents into the oocyte, they degenerate, leaving the quiescent oocyte alone in the follicle before vitellogenesis takes place.

The polytrophic meroistic ovariole is believed to be ancestral to the telotrophic type. The evolution of polytrophic meroistic types from the panoistic form occurred when some cystocytes differentiated into nurse cells that amplified their genome, and incomplete cytokinesis among the cystocytes arose. Telotrophic ovarioles evolved from the polytrophic type when these nurse cells were retained within the germarium. A fourth type of ovariole, the **neopanoistic** type, has secondarily lost its nurse cells by the development of all cystocytes into oocytes or follicle cells. These evolved independently from polytrophic ovarioles at least five times, and they are found in Protura, Thysanoptera, Mecoptera, and Siphonaptera.

VITELLOGENESIS

From the time the egg is laid until the first-instar larva emerges, the embryo must be completely self-reliant. Because it is unable to acquire nutrients or water, the embryo must be supplied with everything it needs for embryogenesis. During this time, a large number of new proteins must be synthesized on a large number of new ribosomes. The ribosomes are acquired from either the increased synthetic capacity of the panoistic oocyte or from the trophocytes in the meroistic oocyte. The major source of nutrients for the oocyte is **vitellogenin**, usually produced outside of the ovary in the fat body, secreted into the hemolymph, and taken up specifically by developing oocytes and deposited in the oocyte cytoplasm as the storage form, **vitellin**. The yolk is broken down and made available for embryogenesis by the vitellophages, those energids that differentiated early during development.

An alternative use of vitellogenins is found in worker bees that do not make eggs even though they are female. The vitellogenin they produce instead binds to their hypopharyngeal glands, and these create the royal jelly used to feed brood. Used as a versatile storage protein that can be adopted by several metabolic processes, the vitellogenin can supplement the metabolism of workers and the synthesis of royal jelly when pollen sources are scarce. Other insects may produce trophic eggs that are fortified with vitellogenins and laid only to be consumed by their immatures.

The bulk of the yolk is protein, mostly large glycolipophosphoproteins that may make up as much as 60% to 90% of the total soluble yolk proteins present. It usually consists of two or more subunits of vitellogenin that are often synthesized as single precursors and cleaved before being exported into the hemolymph. Lipids synthesized in the fat body are also deposited in the oocyte and can make up as much as 40% of the dry weight of an egg. These lipids are mostly triacylglycerol with smaller amounts of cholesterol, phospholipids, and free fatty acids.

They are transported by **lipophorins** through the aqueous hemolymph, which are reusable shuttle molecules that transfer lipid from one tissue to another and are also deposited within the oocyte. A small amount of carbohydrate may be present within the egg as glycogen stores. The yolk is deposited in the oocyte as it descends through the vitellarium, the lower portion of the ovariole, which considerably increases the volume of the oocyte cell. In *Drosophila*, the oocyte volume increases by 100,000 times during vitellogenesis. In *Periplaneta*, this increase in volume is more than 2 million times.

In **autosynthetic vitellogenesis**, found in the primitive apterygotes, the egg synthesizes its own yolk from hemolymph proteins. More advanced insects, as well as vertebrates, engage in **heterosynthetic vitellogenesis**, where the yolk is synthesized elsewhere and transported to the oocyte in the blood. In vertebrates, the liver is stimulated to release vitellogenins into the bloodstream by hormones. In the insects, the fat body is stimulated by hormones to secrete vitellogenins into the hemolymph, and in some flies the follicle cells additionally synthesize and secrete proteins into the oocyte.

The passage of yolk proteins into the oocyte is regulated by the surrounding follicle cells. With **patency**, intracellular spaces appear between the follicle cells to control the uptake of vitellogenin and allow it to be taken up at the oocyte membrane by **receptor-mediated endocytosis** during vitellogenesis. Juvenile hormone regulates this patency by increasing the activity of Na^+/K^+ ATPase in follicle cells. Receptor-mediated endocytosis involves a specific vitellogenin receptor on the cell membrane that recognizes and tightly binds to the vitellogenin and initiates its internalization. The most common endocytotic pathway is **clathrin dependent**. Clathrin accumulates at the site where the receptor binds to the vitellogenin, and the complex is internalized in clathrin-coated vesicles. The clathrin coat is lost, forming endosomes that fuse with one another to form transitional yolk bodies. The vitellogenin and the receptors dissociate, and the receptors are recycled in tubular compartments while the vitellogenin is added to mature yolk bodies as a crystallized form (Figure 4.9). JH also stimulates endocytotic uptake by inducing coated vesicles to fuse.

The genes encoding previtellogenins, the primary products, have been identified for several insects and belong to a family of proteins shared by frogs, chickens, and trout. From one to several genes may be involved; more than one gene may be necessary to produce the large amount of vitellogenin that is required during the short period of egg development for a particular species. The vitellogenins that have been identified so far are encoded by mRNAs of 6 to 7 kb produced by fat body trophocytes and translated into large peptides of about 200 kDa that are subsequently cleaved into subunits from 50 to 180 kDa before being exported from the fat body. The cleavage pattern in hemimetabolous insects is a bit more complicated than in holometabolous ones, where the former produces multiple polypeptides and the latter only two. After various posttranslational modifications, such as glycosylation, the subunits are assembled into the

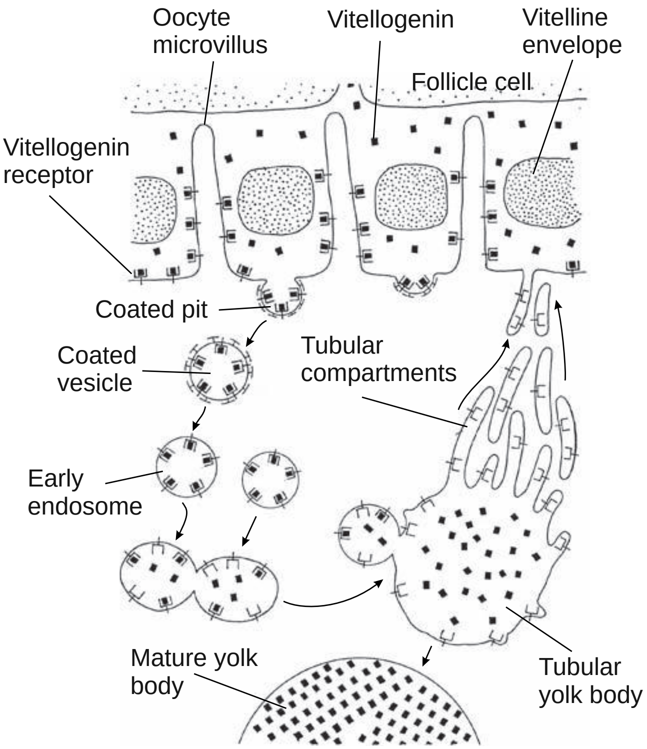

FIGURE 4.9. The process of receptor-mediated endocytosis during vitellogenesis in mosquitoes. The vitellogenin enters from the hemolymph between follicle cells through pores within the vitelline envelope. It binds to receptors on the oocyte plasma membrane, accumulating in clathrin-coated pits that invaginate into the oocyte cytoplasm and form coated vesicles. When the coated vesicles lose their clathrin, they become early endosomes, which fuse to form a larger tubular yolk body. The vitellogenin is crystallized as vitellin, forming a mature yolk body. The vitellogenin receptors are recycled to the plasma membrane by way of the tubular compartments. Modified from Snigirevskaya et al. (1997). Reprinted with permission.

large glycolipophosphoproteins of between 400 and 600 kDa that are secreted into the hemolymph. The 180 kDa primary gene vitellogenin gene product in some higher Hymenoptera is secreted without being cleaved. In the mosquito, *Aedes aegypti*, a 6.5 kb mRNA is translated into a 224 kDa pre-pro-vitellogenin, which is phosphorylated, cleaved into subunits, and the 380 kDa vitellogenin assembled from the subunits (Figure 4.10).

Another unrelated family of egg storage proteins has been identified only from higher dipterans. The three yolk proteins of *Drosophila* are each encoded by single copy genes that are transcribed in both the fat body and follicle cells of the ovary. The three genes, *yp1*, *yp2*, and *yp3*, are located on the X

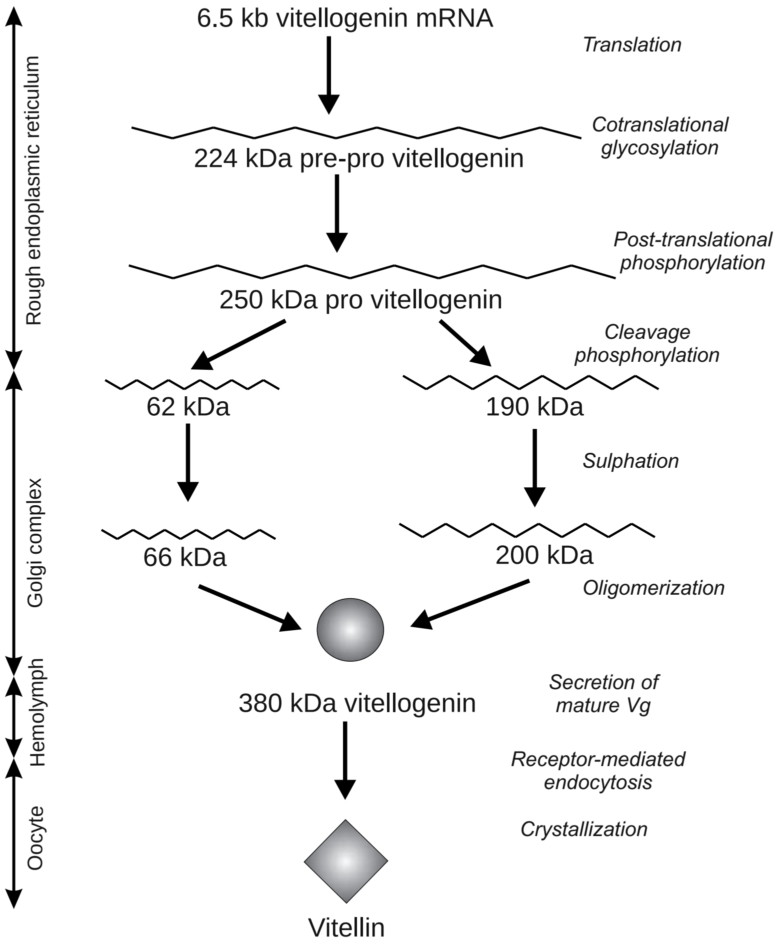

FIGURE 4.10. The processing of vitellogenin mRNA to form the vitellin deposited in the mature yolk body of the mosquito. From Sappington and Raikhel (1998). Reprinted with permission.

chromosome, and the expression of the genes in the fat body is dependent on the gene hierarchy that determines the sex of the individual. In contrast, the expression of *yp* genes in the ovarian follicle cells is developmentally programmed and unrelated to sex or the hormonal environment. Because these yolk proteins made by the follicle cells of *Drosophila* are not lipoproteins, do not circulate in the hemolymph, are evolutionarily unrelated to other insect vitellogenins, and differ in their processing, they have been designated as **yolk polypeptides** rather than vitellogenins. These yolk polypeptides are also present in other higher dipterans including house flies, blow flies, and the tsetse. The tsetse, which matures

only one oocyte at a time, synthesizes a single yolk polypeptide only in its follicle cells. The localization of yolk polypeptide production by follicle cells may better control the development of the one oocyte.

The vitellogenin that is ultimately taken up by the oocyte can be further processed by the oocyte itself to create a crystalline form of the vitellogenin called **vitellin** that is stored within yolk spheres. The vitellin is immunologically indistinguishable from vitellogenin, but it may be different in its solubility and electrophoretic mobility. Other proteins can also be packaged into yolk spheres, including yolk-processing enzymes, hemolymph proteins that are taken up non-specifically, and proteins produced by follicle cells. The oocyte cytoplasm also includes a number of lipid droplets that contain triglycerides and phospholipids, as well as granules of glycogen.

Toward the end of vitellogenesis, the follicle cells produce the **vitelline envelope**, an acellular membrane that surrounds the oocyte. There is evidence that levels of 20-hydroxyecdysone regulate its formation. The last synthetic act of the follicle cells is **choriogenesis**, the secretion of the chorion, the complex lamellar array that was described in Chapter 3. The chorion is an intricate assemblage of proteins that is laid down sequentially to form a semipermeable barrier to the environment. The imprint of each follicle cell is patterned on the surface of the chorion and provides a species-specific signature. The versatility of the follicle cells in producing so many of the products that are necessary for reproduction is truly remarkable. Once the chorion is secreted, this layer forms an impermeable envelope that restricts the movement of substances in or out, with the exception of sperm that enter through the specialized micropyle. When the synthesis of the chorion is completed, the follicle cells degenerate and leave the chorion as the outer surface of the egg.

ENDOCRINOLOGY OF FEMALE REPRODUCTION

Given the tremendous amount of ecological variability among insect species, it is not surprising to find a comparable variability and complexity in the systems that regulate reproduction. Only a few generalizations can be made regarding the endocrine control of female reproduction. In short-lived insects that do not feed as adults, the yolk must be derived from reserves acquired during the larval stage. In these insects, vitellogenesis occurs while the pharate adult is still within the pupal skin and the adult emerges with a full complement of eggs. In the lepidopteran *Hyalophora cecropia*, this preemergence egg development appears to occur in the absence of any identifiable hormonal controls and may simply be a developmental program that is followed by the fat body. Phasmids develop eggs even when the corpora allata are removed, and vitellogenesis appears to be independent of JH. In longer-lived insects that undergo multiple cycles of

reproduction, vitellogenesis occurs when the fat body is activated by hormones that allow the vitellogenins to be produced cyclically.

The specific hormones involved in cyclical vitellogenesis vary considerably among insects, and no single mechanism of hormonal control can be described. However, there are two general approaches to egg production. In some insects, including all hemimetabolous orders, the production of vitellogenins is dependent on JH alone. Insects conforming to this strategy include earwigs, grasshoppers, cockroaches, some lepidopterans, and *Rhodnius*. The few apterygotes that have been examined also show this JH dependence on vitellogenesis. JH acts directly on the fat body cells, causing them to initiate the translation and secretion of vitellogenin and its uptake by oocytes. In the gypsy moth, *Lymantria dispar*, low or declining titers of JH in the last larval instar are necessary for vitellogenin production by the fat body. In those lepidopterans that begin vitellogenesis after adults emerge, including the monarch butterfly, *Danaus plexippus*, and the armyworm, *Pseudaletia unipuncta*, JH also appears to control vitellogenin production.

In other insects, including most dipterans, both JH and 20-hydroxyecdysone are involved. JH regulates the formation of new endoplasmic reticulum in the fat body and the sequestration of the vitellogenin produced, whereas 20-hydroxyecdysone regulates the rate of its production. Variations in the control of vitellogenesis by 20-hydroxyecdysone are common. Vitellogenin production begins as a late pharate adult in the Indian meal moth, *Plodia interpunctella*, and coincides with a decline in the ecdysteroid titer of the pharate adult. In contrast, vitellogenin synthesis in the silkworm, *Bombyx mori*, coincides with a rise of 20-hydroxyecdysone. The reproductive endocrinology of relatively few insects has been examined, and there are many deviations from these generalizations within this small group.

A so-called small PTTH in the silkworm moth, *Bombyx mori*, turned out to be an insulin-like peptide that is now known as bombyxin. Insulin is a hormone commonly associated with glucose metabolism in vertebrates, and the insulin-signaling pathway regulates many aspects of growth and development by signaling nutritional conditions to growing tissues. Currently, 38 genes are known for insulin-like peptides in *Bombyx*, and insulin receptors have been identified on ovarian cells. Genes that encode seven insulin-like peptides with reproductive functions have also been identified in *Drosophila*. Germline stem cell division is stimulated by insulin-like peptides that are produced by both medial neurosecretory cells in the brain and ovarian follicle cells that supplement the signals from within the female stem cell niche. The progression through the stages of *Drosophila* vitellogenesis is also dependent on insulin-like peptide signaling; *Drosophila* that are mutant for the insulin-like receptor (*InR*) are nonvitellogenic and fail to reproduce. *Drosophila* females homozygous for the *chico*^{1} mutation show a reduction in body size and are sterile, although they show normal patterns of JH and ovarian ecdysteroids. The *chico*^{1} gene encodes an insulin receptor substrate

and may block insulin-mediated signaling required for yolk production and uptake. Bovine insulin injected into mosquitoes stimulates ovarian ecdysteroid production that is necessary for the fat body to produce vitellogenin. There appears to be a remarkable conservation of insulin structure and function as a hormone that regulates energy stores and utilization across the animal kingdom.

The control of vitellogenesis in the female mosquito is a good example of the complexity of the mechanisms that have evolved to coordinate reproduction with nutritional state. Because vitellogenesis only occurs in female mosquitoes after a blood meal has been periodically acquired, blood ingestion consequently serves as a method of synchronizing the reproduction of many individuals so that the coordinated events can be better observed. The blood meal provides the precursors for yolk synthesis that are lacking after larval development is completed. After the adult female emerges, the JH that is released by the paired CA during the first few days of life prepare both the fat body and the ovary for vitellogenesis. In response to an early peak of JH, the fat body cells become polyploid to provide more templates for DNA synthesis during vitellogenesis. JH also stimulates the induction of mRNA in fat body cells, the proliferation of its ribosomes, and a development of its responsiveness to 20-hydroxyecdysone. The follicle cells that surround the oocyte-nurse cell syncytium are relatively undifferentiated at emergence, but in response to JH, they begin to differentiate and increase in size. Mediated by endocrine cells dispersed throughout the midgut epithelium, a blood meal releases a pulse of the neurohormone **ovarian ecdysteroidogenic hormone (OEH)** from the brain, which acts on the ovaries to increase their synthesis of protein and stimulate their production of ecdysone, which is converted to the active 20-hydroxyecdysone. The 20-hydroxyecdysone then activates the transcription of genes encoding vitellogenic precursors, vitellogenic carboxypeptidase, and vitellogenic cathepsin B in the fat body. The most abundant fat body transcript is a 6.5 kb vitellogenin mRNA that is translated into a 224 kDA pro-vitellogenin and subsequently cleaved and then repackaged into a 380 kDa vitellogenin (Figure 4.10). Lipophorin, which is commonly used as a transport molecule to shuttle lipid from the fat body to the oocyte, is also deposited in the developing oocyte. In cockroaches, lipophorin protects circulating JH from degradation by JH esterases. The expression of the lipophorin gene during mosquito vitellogenesis is dependent on 20-hydroxyecdysone. The 20-hydroxyecdysone peak also stimulates the follicle cells of the ovary to synthesize the vitelline envelope, the inner layer of the chorion, and acts on the germarium to cause the creation of a new, secondary follicle. During the later stages of egg development, an **oostatic hormone** is produced that prevents the maturation of any secondary follicles until the maturing eggs have been laid, and thus avoids the development of so many eggs that the female could no longer fly.

The promoter region of the mosquito vitellogenin gene contains an ecdysone response element that specifically binds the EcR/USP heterodimer and initiates

a regulatory hierarchy that includes the early genes *AaE74*, *AaE75*, and Broad that encode transcription factors that activate the expression of a set of late genes (Figure 4.11). EcR/USP initiates the activation of the vitellogenin gene while the additional transcriptional regulators act synergistically for full gene expression to occur. There are at least four isoforms of the Broad binding proteins. Whereas the Z2 isoform activates vitellogenin gene expression, Z1 and Z4 repress it (Figure 4.12). The early genes may also repress their own expression through a feedback mechanism. Fat body cells also produce the proenzymes vitellogenic carboxypeptidase and a cathepsin B-like protease that are additionally deposited in the oocyte and are activated during embryogenesis. During vitellogenesis, the additional nuclear receptors regulate the ecdysteroid signaling cascade. The *AaHR38* gene encodes an orphan nuclear receptor that interacts with USP and disrupts its DNA binding capability, repressing vitellogenesis in the absence of 20-hydroxyecdysone before the blood meal. Vitellogenesis is again arrested once egg maturation is completed when the transcription factor Seven-up forms a heterodimer with USP and represses vitellogenesis at that time (Figure 4.13).

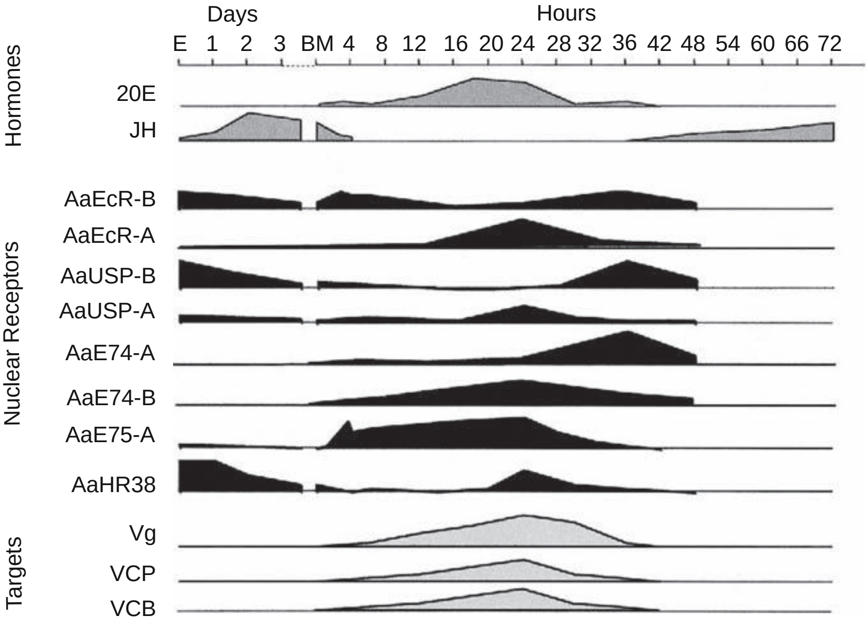

FIGURE 4.11. The appearance of hormones, nuclear receptors, and targets before and after a blood meal (BM) that stimulates vitellogensis in mosquitoes. From Raikhel et al. (2002). Reprinted with permission.

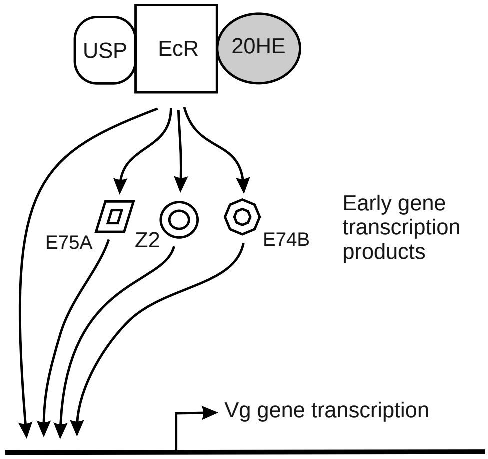

FIGURE 4.12. The regulatory hierarchy involved in the initiation of vitellogenesis in mosquitoes. The heterodimer of USP and EcR that binds to 20-hydroxyecdysone (20HE) causes early gene transcription products to first be expressed. Z2 is an isoform of the Broad transcription factor.

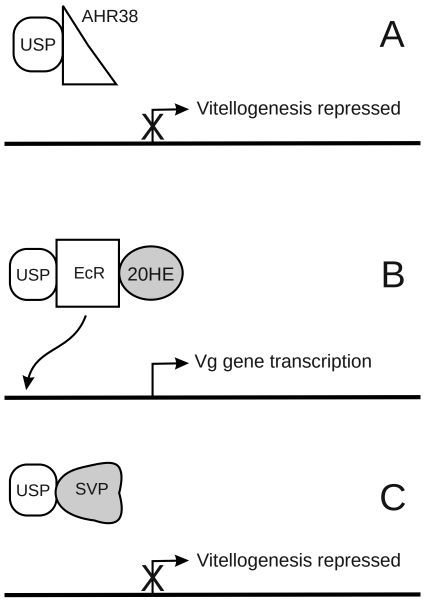

FIGURE 4.13. A. The binding of the nuclear receptor AHR38 to USP in the absence of ecdysone. B. The heterodimer of USP and EcR binds to ecdysone (20HE) and along with other early gene products, stimulates vitellogenesis maximally. C. Once egg development is completed, Seven-up (SVP) forms a heterodimer with USP and represses vitellogenesis until the next blood meal.

OVULATION, FERTILIZATION, AND OVIPOSITION

Once egg development has been completed, the mature eggs are moved into the oviduct by the process of **ovulation**. This is only possible once the follicle cells that surround the oocyte degenerate, leaving the egg free to move out of the ovariole. The muscular contractions of the ovariole and oviduct that propel the egg through the reproductive tract are coordinated by **myotropins** that are secreted by neurosecretory cells when the central nervous system receives a confirmation that mating has occurred and that eggs are indeed mature. For example, in the blood-sucking bug *Rhodnius prolixus*, ovulation is initiated by an FMRFamide-like myotropin that is released only when the spermatheca produces a factor after it is filled with sperm and male accessory gland substances, and the maturing eggs produce 20-hydroxyecdysone. In the female tsetse, *Glossina*, the message that mating has occurred and that stimulates ovulation is not chemical but comes from prolonged mechanical stimulation during copulation.

As ovulation proceeds, the egg traveling down the oviduct triggers receptors that stimulate the spermathecal muscles to contract and release the sperm that are stored within the spermatheca. In *Locusta*, the spermatheca is innervated by three nerves that separately control the anterior and posterior portions. When the anterior portion of the spermathecae contracts, sperm are forced down the spermathecal duct where they are held until sensory cells indicate that the egg has achieved the proper position in the genital chamber. At that point, the posterior duct contracts and moves sperm onto the micropyle of the egg. When the egg has been removed from the genital chamber, the contractions cease. FMRFamide-related peptides are also associated with the *Locusta* reproductive tract and modulate the neurally controlled contractions of the oviduct and spermatheca. The degree of complexity with which the passage of eggs and sperm are coordinated is necessary for their successful union during fertilization.

After they are ovulated and fertilized, the eggs are usually deposited outside of the female's body in the process of **oviposition**. The eggs move down the common oviduct by peristaltic waves of muscular contractions and out of the body through the ovipositor. The movement of the egg downward through the oviducts is facilitated by backwardly directed scales that act like a ratchet mechanism so the egg can only move in one direction, downward toward the genital opening. In many insects, oviposition follows immediately after ovulation, but in some insects the eggs may be retained for variable periods until the eggs hatch or the larvae mature. Some of these variations are discussed later in this chapter (see "Unconventional Methods of Insect Reproduction").

One reason for the diversification and success of insects is their evolution of an ovipositor that allows offspring to be placed and develop in a secure and

appropriate environment that is different from that of the adult. External genitalia in females consist of this ovipositor that can place eggs more precisely in a favorable substrate, but it may have been secondarily lost or reduced in some insects. The ovipositor in most insects consists of two to three pairs of slender valves, the **valvulae**, arising from basal plates, or **valvifers**, on the eighth and ninth abdominal segments (Figure 4.14). A third pair of valves originates from the posterior region of the second valvifers. The valvulae also contain sensory recep-

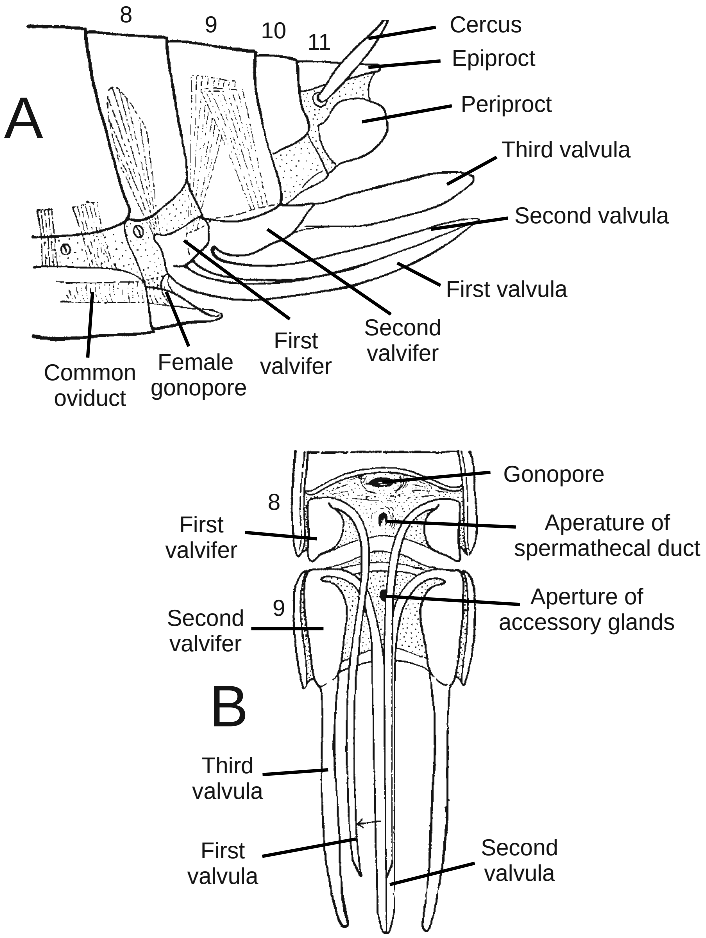

FIGURE 4.14. Components of the ovipositor. A. Lateral view. B. Ventral view. From R.E. Snodgrass (1935). *Principles of Insect Morphology*. Copyright by Ellen Burden and Ruth Roach. Reprinted with permission.

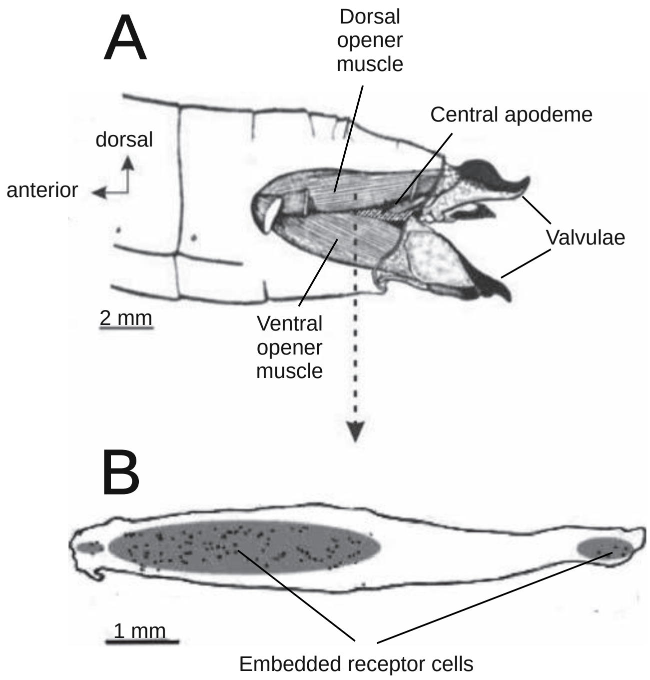

FIGURE 4.15. Embedded tension receptors in a muscle that controls oviposition. A. The terminal segments of the *Locusta migratoria* abdomen showing the location of the muscles that control oviposition. B. A single muscle with its receptors. From Wanischeck and Rose (2005). Reprinted with permission.

tors that evaluate the texture of the substrate and can even perceive light. In *Locusta*, the ovipositor muscles are embedded with tension receptor neurons that modulate digging movements (Figure 4.15). JH controls the coordination of the maturation events of locust ovipositor muscles during the early days of adult female life.

The control of oviposition in the cockroach, *Spodromantis*, is a good example of the integration of environmental and physiological information during egg laying (Figure 4.16). This insect normally lays its eggs at the beginning of the photophase. The brain integrates the information it receives about the photoperiod and the presence of mature oocytes and triggers the release of an oviposition-stimulating hormone that activates the ovipositor, ovariole, and oviduct muscles, and the terminal abdominal ganglion. As the insect probes the substrate with its ovipositor, tactile sensations from sensilla are sent to the terminal abdominal ganglion, which controls further movements of the egg and secretions by the accessory glands.

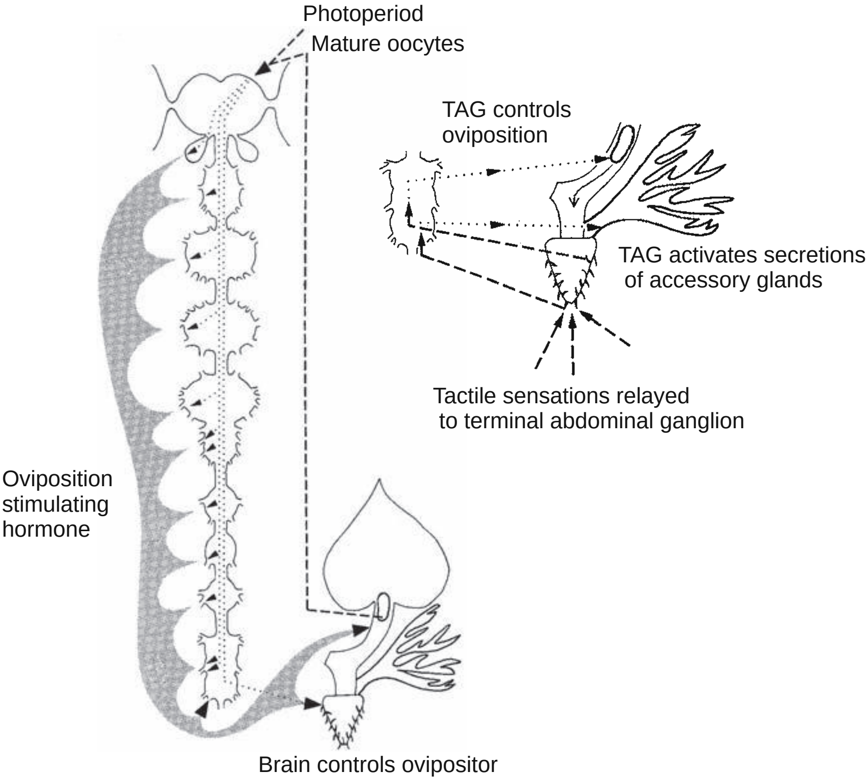

FIGURE 4.16. The control of oviposition in the cockroach, *Sphodromantis lineola*. The proper photoperiod and the presence of mature oocytes are perceived by the brain, which triggers the release of an oviposition-stimulating hormone from the ventral ganglia that causes the oviduct muscles to contract. The brain also controls the searching movements of the ovipositor. Tactile sensations of the ovipositor are relayed to the terminal abdominal ganglion, which activates the secretions by the accessory glands. The perception of all these components by genital receptors causes further contractions of the oviducts and oviposition. From Mesnier (1984). Reprinted with permission.

MALE REPRODUCTIVE SYSTEMS

Spermatozoa are produced within the paired **testes** of the male (Figure 4.17). Each testis is composed of a series of tubular **follicles**, which can vary in number from one in some apterygotes and dipterans to more than 100 in Orthoptera, and up to 300 in Hymenoptera. The follicles are in turn enclosed by a **peritoneal sheath**. More primitive insects have a single testis, and in some lepidopterans the two maturing testes are secondarily fused into one structure during the later stages of larval development, although the ducts still remain separate. Within the follicles the developing sperm are in successive stages of maturation. The follicles connect to a main duct, the **vas deferens**, through individual **vas efferens** tubes (Figure 4.18). A portion of the vas deferens may be enlarged as

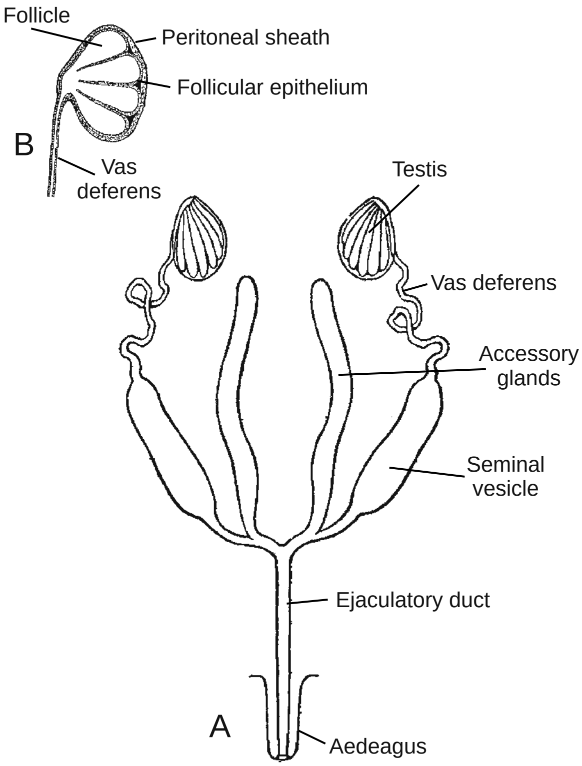

FIGURE 4.17. A. The generalized male reproductive system. B. A cross section of the testis. From Snodgrass (1935). Reprinted with permission.

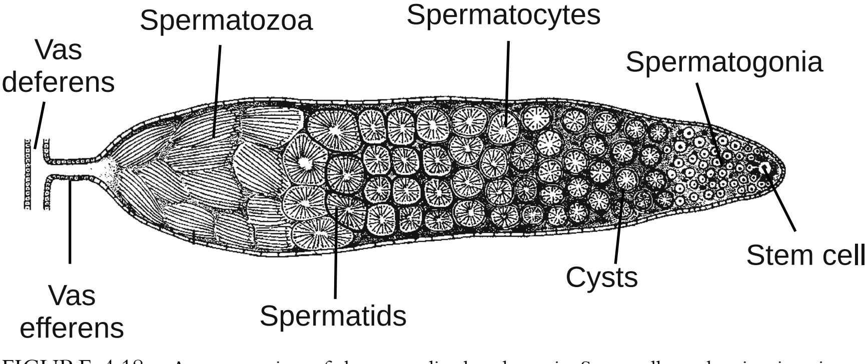

FIGURE 4.18. A cross section of the generalized male testis. Stem cells at the tip give rise to spermatogonia by mitotic division and become enclosed by somatic cells that produce the cysts. Incomplete mitotic divisions of the spermatogonia form the spermatocytes that are connected by ring canals. The spermatocytes divide meiotically to form the haploid spermatid, which then differentiates into flagellated spermatozoa. As they mature, they rupture the cyst wall and escape to the seminal vesicles. Modified from Chapman (1982). Reprinted with permission.

the **seminal vesicle** that serves as a storage reservoir for sperm before they are transferred to the female. The two vasa deferentia are surrounded by circular muscles and connect to the **ejaculatory duct**, which is composed of cells that are ectodermal in origin and produce a lining of cuticle.

The terminal portion of the ejaculatory duct may be sclerotized to form the intromittent organ, the **aedeagus**. It differentiates from a pair of ectodermal lobes associated with the ninth abdominal segment and is often concealed within a genital chamber (Figure 4.19). Other accessory structures, often known as **claspers**, may be present on neighboring segments and adapted for grasping the female during copulation. An aedeagus may be absent in Apertygota, and in Odonata there is a secondary copulatory structure at the anterior of the abdomen that performs its function. Some more primitive pterygotes have developed a pair of intromittent organs. The male members of one family of earwigs that have paired intromittent organs use the spare when one is damaged during mating.

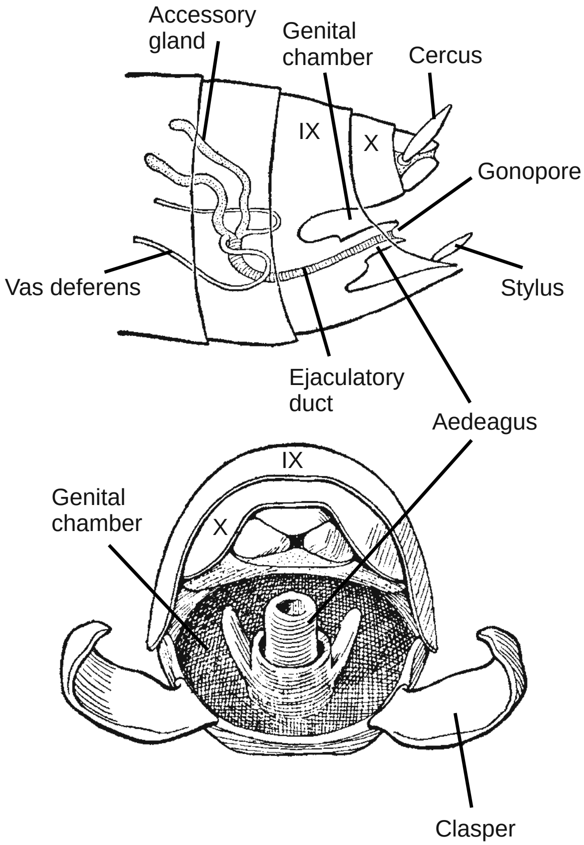

FIGURE 4.19. The generalized male terminalia. From R.E. Snodgrass (1935). *Principles of Insect Morphology*. Copyright by Ellen Burden and Ruth Roach. Reprinted with permission.

A pair of male accessory glands is also typically present. These glands can open either into the vas deferens or the ejaculatory duct. Those arising from the vas deferens during development are mesodermal in origin, whereas those originating from the ejaculatory duct have an ectodermal origin. Male accessory glands serve a variety of functions, including the production of the **seminal fluid** that serves as a transport and activation medium for sperm, the **vaginal mating plug** that temporarily blocks sperm from another male from entering, and the formation of **spermatophores** that are proteinaceous secretions of the male accessory glands that enclose the sperm. Like all animals, insects descended from aquatic ancestors, and the synthesis of a spermatophore is associated with the evolutionary transition to the terrestrial habitats they have come to occupy. Internal fertilization is most appropriate for animals that live on land, and the spermatophore may be a transitional form that represents an initial adaptation for life on land to protect the male gametes from desiccation until they are in the female reproductive tract. Apterygote males produce a spermatophore that is deposited on the moist ground and then is taken up by the female. In more advanced insects, the sperm are transferred directly in seminal fluid by internal fertilization, and spermatophores are not produced.

Peptides that are produced by the male accessory glands and transferred to the female during mating can affect several physiological systems of the female. A common effect is the prevention of subsequent mating by the female, either temporarily or permanently. Much like a nuptial gift, male accessory gland substances can also supplement the nutritional reserves of the female and allow her to increase her egg production when mated. Unmated females of many insects are incapable of laying the eggs that may develop, and the components in male accessory glands are able to remove the physiological block that prevents oviposition until mating takes place. The circadian rhythmicity of females can be altered by mating, and this alteration is commonly mediated by male accessory gland substances. Male *Drosophila* include antibacterial proteins in their accessory gland secretions that may protect the sperm, as well as the male and female reproductive tracts, from infection.

Spermatogenesis

Male gametogenesis in insects has remarkable similarities to that in mammals. In both groups, a small number of stem cells continue to divide, giving rise to spermatogonial cells and the renewal of the stem cells. The spermatogonia divide first mitotically and then meiotically to yield primary spermatocytes that differentiate into spermatids.

The production of spermatozoa occurs within the follicles of the testes. At the anterior end of the testes are apical stem cells that are flanked by specialized nondividing somatic cells, called the **hub**, that form the stem cell niche. Signals

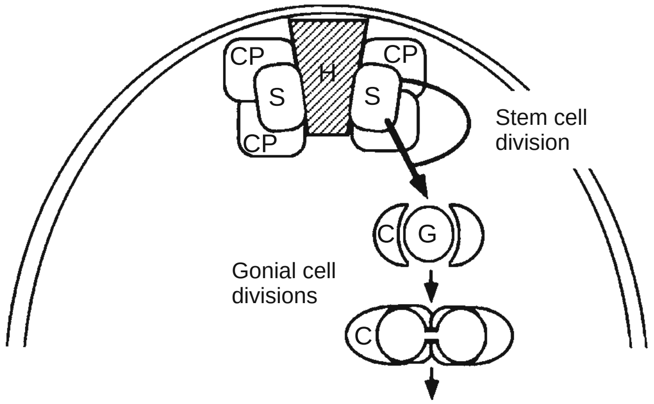

FIGURE 4.20. The male stem niche. Stem cells (S) surround the hub (H), dividing to form a gonialblast (G). Cyst progenitor cells (CP) give rise to new cyst cells (C) that enclose the gonialblast to form the follicle. From Fuller (1998). Reprinted with permission

from the niche control the behavior of the stem cells and regulate their renewal, maintenance, and survival. Stem cells are located around the hub, and with niche signaling they divide mitotically to produce one **gonialblast** and another stem cell (Figure 4.20). The somatic **cyst progenitor cells** of the hub give rise to new **cyst** cells and progenitor cells. The cyst cells enclose the gonialblast to form a **follicular cyst**. Once enclosed, the *Drosophila* gonialblast initiates four mitotic divisions with incomplete cytokinesis, resulting in 16 spermatogonia connected by ring canals. As in the cystocytes of the female, a fusome is present in each of the cells, spilling over into the ring canals, but the ring canals in the two sexes of *Drosophila* differ in their structural components. The ring canals of the male system are lined with **anillin**, an actin-binding protein that attaches it to the membrane.

At the 16-cell stage in *Drosophila*, the spermatogonia initiate meiosis to become primary spermatocytes and grow in volume by about 25-fold. The number of divisions within a cyst is species specific; in *Rhodnius*, the spermatocyte stage is reached at eight divisions, or 128 cells per cyst. Gene expression begins in this stage, mostly of those genes encoding proteins that are required to complete spermatogenesis. Meiotic division yields haploid spermatids, and the gene transcription ceases. The spermatids then differentiate into spermatozoa, via the sequence of developmental events within spermatogenesis called **spermiogenesis**, utilizing the proteins already produced (Figure 4.21). However, spermatid differentiation is independent of the meiotic divisions; cell cycle mutants that fail to divide meiotically still undergo differentiation. The events of differentiation include the development of the microtubules and the elongation of the **flagellum**, a condensation of the DNA accompanying a change in nuclear shape,

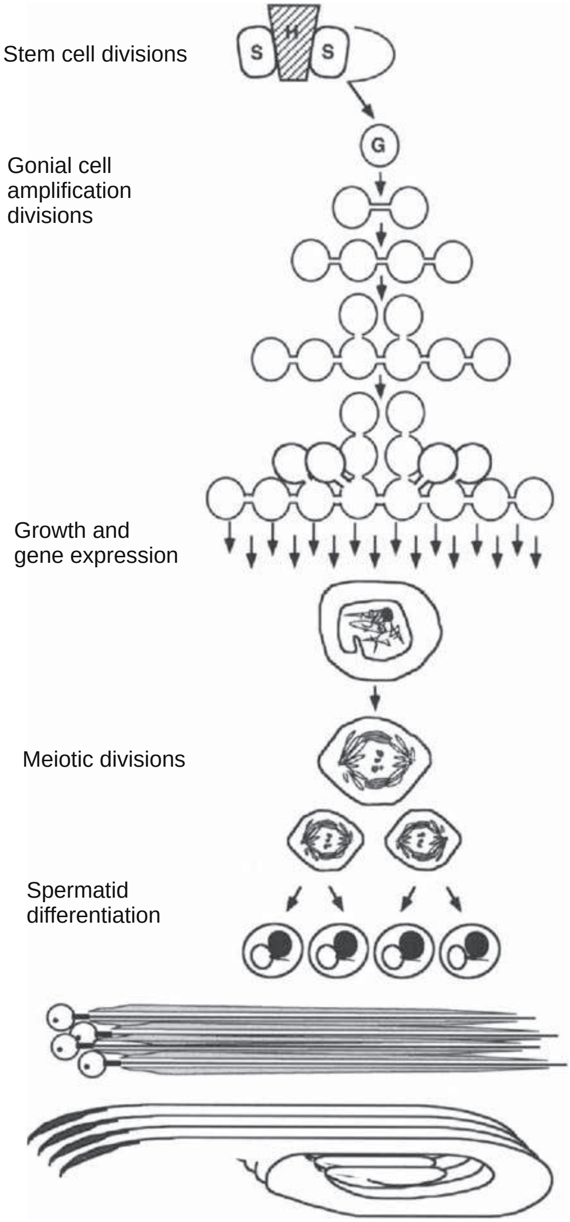

FIGURE 4.21. The process of spermatogenesis in *Drosophila*. Shown is the stem cell niche containing the hub (H) and stem cells (S). A stem cell divides mitotically to form the gonialblast (G) that undergoes incomplete cell division to form spermatogonia connected by ring canals. Spermatogonia divide meiotically to form spermatids that subsequently differentiate into spermatogoa. From Fuller (1998). Reprinted with permission.

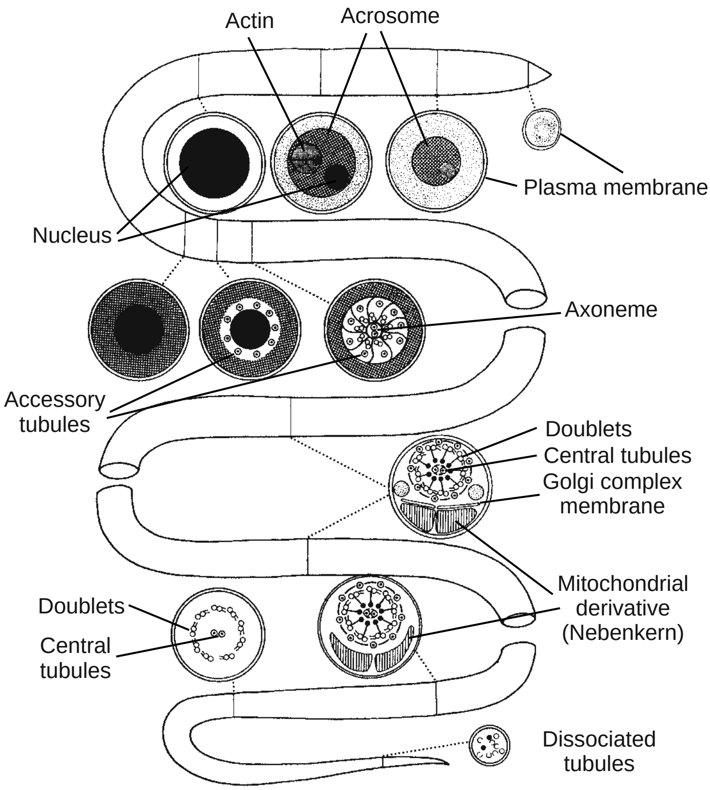

FIGURE 4.22. A cross section along different locations of a generalized insect sperm. From Baccetti (1998). Reprinted with permission.

and the formation of a specialized mitochondrial derived structure, the **Nebenkern**, that is also associated with the flagellum (Figure 4.22). The **acrosomal complex** develops at the sperm head as a skeletal glycoprotein associated with the enzymes acrosin and hyaluronidase.

A number of genetic mutants have been identified that interfere with spermatogenesis. Fusion of the mitochondria to form the Nebenkern requires the gene *fuzzy onions* (*fzo*). Males mutant for *bag of marbles* (*bam*) are unable to transition from spermatogonia to spermatocytes. Mutants for *no mitochondrial derivative* (*nmd*) undergo flagellar elongation without the presence of the mitochondrial derivative. The genes *always early* (*aly*), *cannonball* (*can*), *meiosis I arrest* (*mia*), and *spermatocyte arrest* (*sa*) are necessary for the progression through meiosis and eventual spermatid differentiation. In *twine* mutants, some events of meiosis are skipped, but differentiation into spermatids still occurs.

As differentiation proceeds, the cysts elongate and eventually rupture, releasing the spermatozoa into the vas efferens. These spermatozoa move to the seminal vesicles, effected by the contractions of the walls of the reproductive tract, where they remain until mating takes place. In Lepidoptera, this migration occurs before adult emergence and depends on a circadian clock located in the reproductive tissues that produces a two-step release, first to the vas deferens and then to the seminal vesicles. The circadian clock gene, *period*, is expressed in the epithelium of the vas deferens. In the gypsy moth, *Lymantria dispar*, the sperm descend from the testes into the reproductive tract 4 days before the adult ecloses.

Spermatozoa

Insect sperm are morphologically similar to those of vertebrates, containing a head region and a long flagellum that is used for locomotion (Figure 4.22). Sperm are the only cells of the insect that bear flagella, but even these may be absent in proturans whose sperm are disc-like (Figure 4.23). Within the head are a haploid nucleus and the **acrosomal complex** at the tip that arises from the Golgi apparatus during differentiation. The acrosome contains the enzyme **acrosin**, a trypsin-like enzyme that dissolves the egg membranes for fertilization. The motor portion of the flagellum is called the **axoneme** and is composed of microtubules originating from the centriole at the base of the sperm nucleus. The sperm of some primitive apterygotes have no flagellum at all, but most pterygote sperm have a flagellum with a 9 + 9 + 2 arrangement of microtubules (nine outer accessory tubules, nine doublets, and two central tubules), which differs from the classical 9 + 2 arrangement in other animals with cilia or flagella.

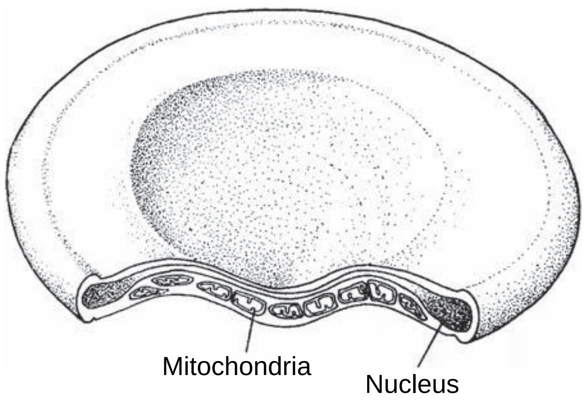

FIGURE 4.23. A disclike spermatozoa of proturans. From Baccetti (1998). Reprinted with permission.

With few exceptions, the nine accessory tubules are additionally found in the Diplura and beyond. The microtubules are made of the dimeric protein **tubulin**, with arms consisting of the contractile protein **dynein** that forms cross-bridges between the microtubule fibers and allows them to slide past each other to effect bending of the flagellum (Figure 4.24).

The tail length of flagella may be variable even within a species; in *D. melanogaster*, the sperm range in length from 1.6 to 2 mm, but *D. bifurca* males produce sperm with a total length of over 58 mm, the longest described for any animal and many times the length of the adult fly that produces them. Males of

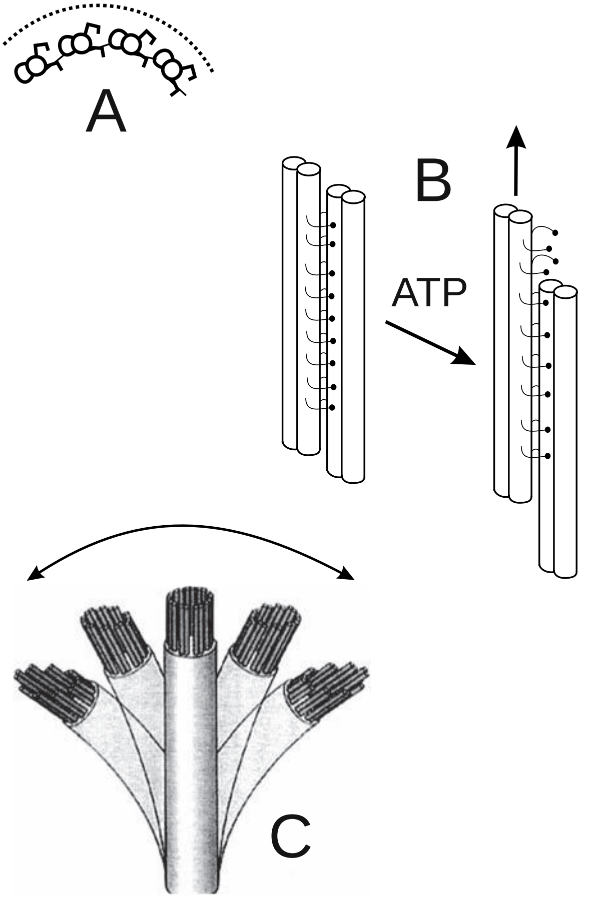

FIGURE 4.24. The mechanism of sperm flagellum movement. A. Cross section of microtubular cytoskeleton. B. Tubulin microtubules with dynein arms form and break cross-bridges with ATP. C. When their bases are stationary, the microtubules slide past each other, causing the assemblage to bend.

all species within the *Drosophila obscura* group produce at least two different sperm lengths, and four different sperm lengths ranging from 69 to 430 μm are produced by *Drosophila subobscura*. Individual males of some anopheline mosquito species produce polymorphic sperm that vary widely in length from 50 to over 500 μm. *Drosophila* that produce longer sperm tend to also take longer to become reproductively mature.

Lepidopteran males are unusual in that all but the most primitive species produce two very different types of sperm in their fused testes. Both types are transferred to the spermatheca of the female, but only the conventional **eupyrene** sperm are involved in normal fertilization of the oocyte. The other **apyrene** sperm have no nuclei, losing them at a late stage of spermatogenesis, and thus cannot have any genetic function. Actin filaments on the sperm bundles produce peristaltic contractions that squeeze both types out of their cysts. Eupyrene sperm differentiate during the larval stage, but apyrene do so later after the larvae initiate spinning. In the absence of any genetic role, the function of apyrene sperm may be to assist in moving the eupyrene sperm through the female reproductive tract or provide them with nutrients. Apyrene spermatozoa are generally shorter than eupyrene sperm, are initially more motile, and are present singly in the seminal vesicles when the eupyrene sperm are still in bundles, gaining their motility when they are placed into the spermatophore (Figure 4.25). As many as 70% to 90% of the spermatozoa transferred may be

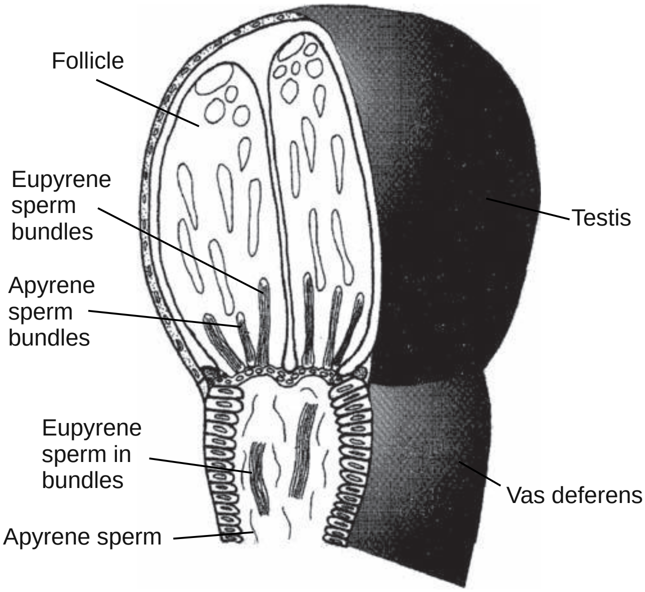

FIGURE 4.25. The fused testis of lepidopterans and their production of bundles of apyrene and eupyrene sperm. From Giebultowicz et al. (1997). Reprinted with permission.

apyrene, and they may displace the eupyrene sperm that are already present in the spermatheca from the previous matings of the female. Both types are necessary for fertilization to occur; artificial insemination of female *Bombyx mori* with only eupyrene sperm fail to fertilize eggs unless apyrene sperm are added.

Endocrine Control of the Male Reproductive System and Spermatogenesis

Relatively little is known about the endocrine regulation of male reproduction compared to what is known about the female. In short-lived insects that may not feed as adults, spermatogenesis occurs early during the larval and pupal stages. In longer-lived males, spermatogenesis continues throughout adult life. With the vastly different hormonal conditions that exist during the immature and adult periods, a unifying scheme for the control of spermatogenesis has not been possible. Indeed, it has been suggested that insect spermatogenesis may simply be a sequential process of differentiation that is completely independent of hormones. The testes of several lepidopterans produce ecdysteroids at the end of the last larval instar and during pupal development and also contain ecdysteroid receptors that regulate the transcription of target genes. The testes are induced to synthesize the ecdysteroids when stimulated by an **ecdysiotropin** produced by the medial neurosecretory cells of the brain. This ecdysiotropin is different than PTTH and fails to act on the prothoracic glands; its activity appears to be limited to the testes. A testis ecdysiotropin is also produced by the brain, subesophageal ganglion, and the testes sheath of male *Rhodnius*.

The rate of mitotic divisions of spermatogonia to form spermatocytes is increased by high levels of 20-hydroxyecdysone, but high titers of JH abolish this increase. The spermatocytes then begin meiotic divisions that are arrested at prophase until the end of the larval period. The postwandering peak of 20-hydroxyecdysone unblocks meiosis and allows the cells to proceed to metaphase. In some insects, JH accelerates spermatogenesis. The release of mature spermatozoa from the cysts in the testes displays a circadian rhythmicity that is initially inhibited by 20-hydroxyecdysone. The decline of 20-hydroxyecdysone is thus necessary for sperm to be released.

Spermatogenesis is interrupted in those lepidopterans that undergo a larval or pupal diapause but resumes once diapause has been completed. It is not any developmental activity that causes this interruption but rather the lysis of developing gametes before they become mature. The renewal of spermatogenesis occurs with increasing titers of 20-hydroxyecdysone that occur when diapause is terminated. In lepidopterans, the differentiation of apyrene sperm from eupyrene sperm occurs with the exposure to a hemolymph-borne apyrene-spermatogenesis-inducing factor.

Male accessory gland function is regulated by several hormones, but JH has the most prominent role in stimulating protein synthesis in the gland. Stimulation of protein synthesis has also been reported by 20-hydroxyecdysone in some lepidopterans and by brain neuropeptides in *Rhodnius prolixus*. The glands are also a source of JH. Indeed, the first extracts of JH for experimental use in the 1950s came from the male accessory glands of *Hyalophora cecropia*. This JH may also be transferred to the female during mating to enhance vitellogenesis.

UNCONVENTIONAL METHODS OF INSECT REPRODUCTION

In most insects, females mate with males, fertilize the eggs just before oviposition, and lay them outside of the body. This most common means of reproduction is termed **oviparity**. There are also some more unusual methods of producing offspring among the insects.

Parthenogenesis

Sexual reproduction involves the fusion of the two haploid nuclei from the sperm of the male parent and the egg of the female parent (Figure 4.26). Occurring in most insects, this sexual reproduction offers an opportunity for the reassortment of genetic information that provides the phenotypic variation that is acted upon by natural selection. However, there are examples of **parthenogenesis**, or the development of unfertilized eggs, in almost every insect order. Unlike sexual reproduction, parthenogenesis no longer provides any opportunity for the reassortment of parental genes. The populations that reproduce parthenogenetically thus have much greater genetic stability, but this stability can be a disadvantage in a changing environment because it reduces the amount of variation on which natural selection can operate. With a drastically changing environment, a parthenogenetic population can be wiped out because it has no phenotypic variation to fall back on. However, there are advantages to parthenogenesis that allow it to be maintained in a population. With fertilization unnecessary, parthenogenetically reproducing females do not have to expend energy to find members of the opposite sex. Allocation of resources to devote to pheromone production or swarming is not necessary. When a female is no longer required to call attention to herself to attract a male, the risk of predation from making oneself more obvious in general is also reduced.

Another potential difficulty in parthenogenesis is that with fertilization absent, the haploid gametes fail to unite, so the diploid number of chromosomes in the zygote must be restored in some other way. The mechanism of restoration can be classified as either **haploid parthenogenesis** or **diploid parthenogenesis**.

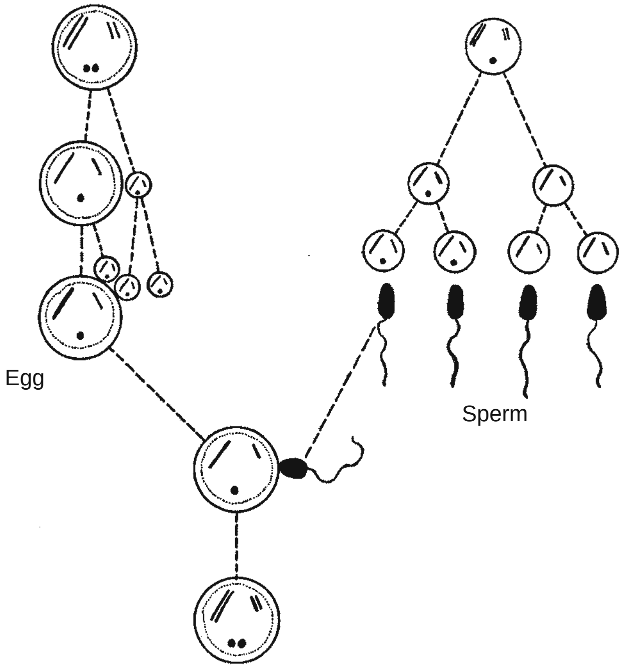

FIGURE 4.26. The formation of sperm and eggs by meiotic division, and the fertilization of the egg to produce a diploid zygote by sexual reproduction. From Johannsen and Butt (1941). Reprinted with permission.

In haploid parthenogenesis, or **haplodiploidy**, meiosis by the oocyte produces a reduction in chromosome number that results in haploid gametes, and the haploid eggs may develop with or without fertilization. The mother is able to control the sex of her offspring by controlling the release of sperm during ovulation and oviposition. Eggs that are fertilized become female; those that are not fertilized remain haploid and become male (Figure 4.27). Although the cytology of oogenesis is normal, spermatogenesis is not, because the haploid males must also produce haploid sperm and therefore lack the meiotic divisions in their formation. In diploid parthenogenesis, the parthenogenetically developing eggs are diploid, and the diploid number is regained from haploid gametes in several ways. In **automictic parthenogenesis**, the early stages of meiosis are normal and the chromosome complement in the egg is haploid. The diploid number is restored not from the fertilization by a spermatozoan (**amphimixis**) but by fusion of the egg nucleus with a polar body so that two nuclei from the same individual fuse. Because most insects have a system of sex determination in which the female has two X chromosomes, this form of parthenogenesis produces all females (Figure 4.28). In the Lepidoptera where the female is XY, reproduction

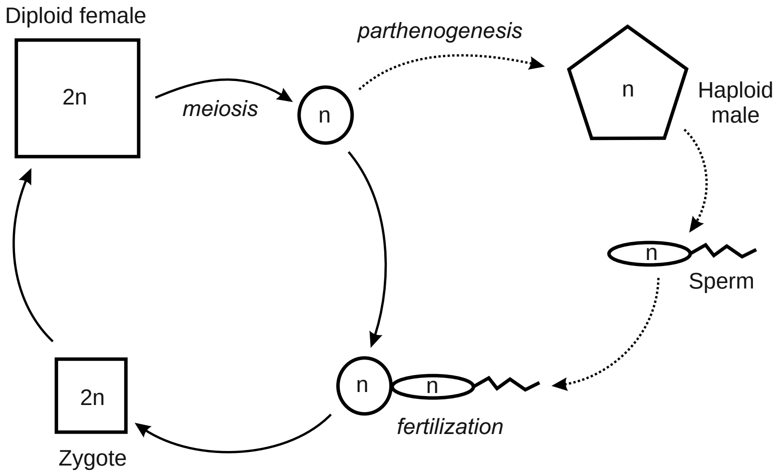

FIGURE 4.27. An example of haploid parthenogenesis. The diploid female produces haploid eggs by meiosis; if the eggs are fertilized, they become a diploid zygote that develops into a female. Unfertilized eggs develop into haploid males.

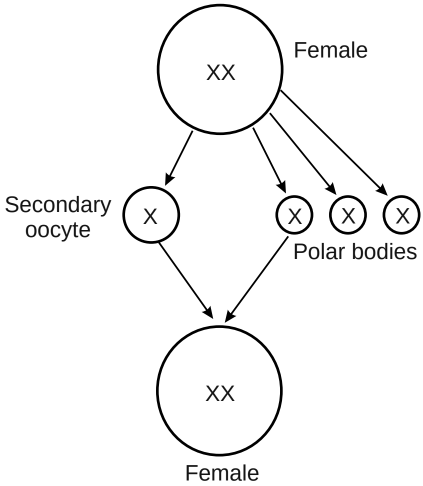

FIGURE 4.28. Automictic parthenogenetic development, in which the oocyte fuses with a polar body.

by automictic parthenogenesis may produce either a male or a female (Figure 4.29). Another form of diploid parthenogenesis is probably more common in insects. In **apomictic parthenogenesis** there is no problem in restoring the diploid number because the oocyte does not undergo a reduction division. The oocyte remains diploid and the egg develops in the normal way. With meiosis

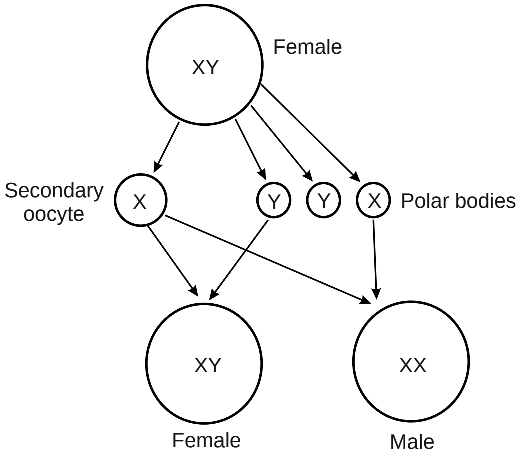

FIGURE 4.29. Automictic parthenogenetic development in Lepidoptera, where the mechanism of XY sex determination produces polar bodies that may have either an X or Y chromosome, producing either male or female progeny.

absent, offspring retain the genetic constitution of the mother. The trigger for further egg development in the absence of sperm penetration and fusion as a trigger may come from the displacement of the oocyte nucleus as it is squeezed through the oviduct during oviposition.

Viviparity

Most insects are **oviparous**, developing their eggs internally and fertilizing them just before oviposition, with the eggs hatching after being laid outside of the female. **Viviparity**, in contrast, involves the retention of eggs for varying periods after they have been fertilized, allowing embryonic development to be completed within the female. The eggs hatch while inside the female, and larvae are deposited instead of eggs.

There are four main types of viviparity. In **ovoviviparity**, the eggs contain sufficient yolk to nourish the embryo until it hatches and are retained by the female without her providing any nourishment. The female deposits the larvae soon before or immediately after they hatch. In **adenotrophic viviparity**, the embryo develops within the female, but when the egg hatches, the larva is retained in the modified genital chamber and feeds from secretions of the female accessory gland. The larva molts within the female and is deposited as either a mature larva or a pupa. In **hemocelous viviparity**, the ovaries lie free within the fat body and disperse eggs within the hemocele. The eggs are surrounded by a group of specialized follicle cells, the **trophamnion**, that feeds the oocyte

from maternal tissues. Mature larvae either escape through a brood canal, as in Strepsiptera, or devour the tissues of the maternal larva, as in some cecidomyiid dipterans. In **pseudoplacental viviparity**, the eggs have little yolk, and the developing embryo receives nourishment through a special structure formed from follicle cells, the **pseudoplacenta**, that absorbs nutrients from "milk" secreted by the female accessory glands.

Polyembryony

In **polyembryony**, found in some parasitic Hymenoptera, one sexually produced embryo is split into up to several thousand others during development after the egg is oviposited. It thus shares some of the characteristics of both sexual reproduction and parthenogenesis. Unlike parthenogenesis that produces many copies of a single genotype, the genotypes of the mother and offspring differ in polyembryony. Unlike sexual reproduction, however, all the genotypes produced are the same as each other. Occurring after the egg is oviposited, polyembryony enables the offspring, rather than the parent, to determine optimal brood size.

An accidental form of polyembryony, or twinning, where an individual egg may split to form two or more embryos, has been observed in most animals, but an obligatory form is rare. Obligatory polyembryony occurs in four families of parasitoid wasps. The 2-mm long wasp *Copidosoma floridanum* lays a single tiny egg into the egg of a host moth. The host completes embryogenesis and hatches into a first instar larva, and during the four host instars, the *Copidosoma* embryo proliferates. At the fifth larval instar, up to 2000 larvae of *Copidosoma* are formed and begin to consume the host, ultimately forming a mummy. The wasp larvae pupate and then emerge through a hole in the host cuticle.

The single wasp egg that is laid in the egg of a lepidopteran host has no yolk and is surrounded by a thin chorion. The wasp exploits the nutritive environment of the host both for embryogenesis as well as for larval development. Unlike the syncytial cleavage characteristic of most other insects, it undergoes holoblastic cleavage soon after oviposition and develops in a completely cellularized environment. In the absence of the syncytium, there is also no opportunity for the distribution of patterning morphogens within the developing egg, and specification of developmental axes occurs later in each embryo. The segmentation proteins commonly found during *Drosophila* embryogenesis are expressed later in development.

Paedogenesis

Some insects are able to engage in reproduction as larvae. This form of reproduction is termed **paedogenesis** and is necessarily parthenogenetic because the

larvae have no external reproductive structures for mating. The best example of this is the dipteran *Miastor*, which produces eggs from ovarioles dispersed throughout the larval fat body. Under inadequate nutritional conditions, the developing embryos at first absorb nutrients from the maternal fat body, but after hatching, the larvae feed on the mother's internal organs and ultimately kill her. The larvae escape through the cuticle and soon initiate another paedogenic cycle. Paedogenesis also commonly occurs in aphids when they reproduce parthenogenetically. When the development of offspring begins in the parent before the parent has reached adulthood, their development is considered to be paedogenic.

Hemocelic Insemination

There is a bizarre method of insemination in the hemipteran superfamily Cimicoidea. In many members of this group, the males do not copulate using the genital opening but instead puncture the integument of the female with their sharp intromittent organ. They inject their sperm and semen into the hemolymph, sometimes in specialized integumental structures in the hemocele rather than introducing sperm conventionally into the genital tract. Males can even "taste" whether the female has recently copulated and if she has, they reduce their duration of copulation and ejaculate size. A progression toward this specialized insemination can be seen within species in this hemipteran superfamily. In the nabid, *Alloeorhynchus*, the injection of sperm occurs through the wall of the female's genital tract. A spine on the penis ruptures the genital wall, and sperm move through the hemolymph and collect around the ovarioles where they fertilize the eggs. Some of the sperm that enter the hemocele are phagocytized by hemocytes and may be used as nutritional precursors by the female. In *Primicimex,* a bedbug found in bat caves, sperm are injected into the body cavity of the female through the abdominal integument. The number of sclerotized scars on the female's cuticle is a good indication of the number of times she has mated. The sperm circulate within the hemolymph and accumulate in pouches at the base of the oviducts. The genital tract of the female is used only for oviposition and not for copulation. In the bedbug *Cimex*, which is highly specialized for hemocelic insemination, a cuticular pouch, the **ectospermalege**, has evolved as a special reception site (Figure 4.30). A group of mesodermal cells, apparently derived from hemocytes, forms a **mesospermalege** that phagocytoses some of the sperm and also conducts others to the base of the oviducts. Sperm migrate to the ovaries where fertilization takes place. This type of insemination was considered to be beneficial to the female by allowing her to absorb some of the nutrients in the spermatozoa and seminal fluid and use them for egg maturation. However, traumatic insemination may be more of an evolutionary response to a sexual conflict of interests. The coercive copulatory strategy by the male is

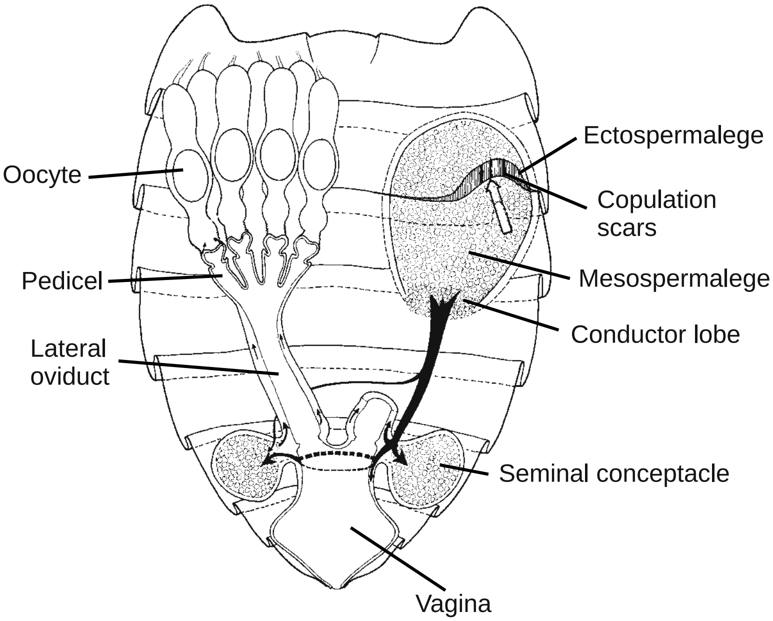

Figure 4.30. The abdomen of the bed bug, containing the reproductive tract. The ectospermalege, a cuticular pouch on the abdomen, is a special reception site for sperm introduced by hemocelic insemination. From Carayon (1966). Reprinted with permission.

countered by the evolution of the paragenital system in the female that reduces the potential effects of wounding and allows the female to exercise some control over which sperm fertilize the eggs. Females receive no apparent benefits from multiple mating but do show reduced longevity and reproductive success.

MATING SYSTEMS

During the process of natural selection, certain traits an individual possesses are affected by climate, predators, and competition with other species, and this interaction can determine its survival and the number of descendants it produces during its lifetime. **Sexual selection**, a category of natural selection, presumes that the survival of particular genes within the gene pool can be affected by a selection for certain traits that increase the chances that an individual will be successful in acquiring mates. The number and quality of mating encounters, and the resulting number of offspring produced, can be influenced by the ability of males to compete among themselves for females or for their capacity to attract females. Sexual selection can also occur after sperm have been introduced in what has been called **cryptic female choice**, where the female selects for more

cryptic male traits that are not associated with the classical measures of male mating success. Variation in the features of sperm, such as flagellum length and the size of the enzyme-containing cap that aids in the penetration of the egg, can provide an advantage over other sperm in their competition for fertilizing the egg. Females can potentially store sperm from several partners in their spermathecae, and the possibility exists for competition between the sperm that are present to be used for fertilization. This **sperm competition** is a type of cryptic female mate choice in which females exercise a differential acceptance of sperm from males of varying quality when multiple mating occurs. For example, female moths and butterflies are able to select for the larger-sized sperm produced by certain males after insemination takes place, using these to fertilize eggs. Sperm competition as a mechanism of cryptic female choice is an effective force of sexual selection that generates a myriad of adaptations that aim toward ensuring that certain sperm are used for fertilization. This sexual selection by females can influence the paternity of offspring by discriminating not only between males before copulation occurs but also by choosing among sperm after copulation has taken place.

Because sperm are stored in the female's spermathecae after copulation, the process of insemination is usually temporally distinct from the fertilization of eggs. Sperm may be primitively transferred to the female within a spermatophore, which is deposited on the ground by some apterygotes, or into the gential tract of the female as by primitive pterygotes. Sperm migrate from the spermatophore into the spermatheca, and the empty spermatophore may be ejected, eaten, or resorbed by the female. In many more advanced insects, direct insemination of the female occurs when the male deposits the sperm in seminal fluid into the genital tract or directly into the spermatheca. Sperm may be stored within a spermatheca for long periods of a year or more and may be mixed with the sperm of several males. The positioning of sperm within the spermatheca may give certain types a selective advantage when the female releases them for fertilization.

Males engage in several strategies to protect their genetic investment and prevent other males from mating. **Sperm precedence** dictates that the sperm that are present from the most recent matings may be the ones that are most likely to be released to fertilize the egg. This differs from the practice in vertebrates that lack a sperm storage organ and use the sperm from the first inseminator for fertilization. Males of the giant water bug, *Belastoma*, accept the eggs from a female and incubate them on their backs, but they mate repeatedly with a female before and during her oviposition to assure their paternity. Some odonate males also guard their mates after inseminating them, instead of searching for other females to mate with, to prevent them from receiving other sperm. The penis of the male damselfly is fitted with hairs and projections that can remove the existing sperm in the female's spermatheca before adding his own, thus assuring his paternity. A male can remove competing sperm by **sperm**

displacement in which his own ejaculate can overwhelm and flush out already existing sperm. It is to the advantage of the male to produce the largest quantity of sperm, and he is often able to adjust the quantity as conditions warrant. Males tend to ejaculate more sperm when competing males are present than when they are alone with a female. In bedbugs that engage in traumatic insemination, a male can use his penis to detect whether the female has already been inseminated and will subsequently reduce his own contribution when competing sperm are present.

Rather than form a spermatophore, male accessory gland substances may produce other components to increase the reproductive success of the male. Male accessory gland secretions may create mating plugs that temporarily delay subsequent inseminations, or they may act chemically as pheromones to make the female physiologically refractory to subsequent males and establish the precedence of sperm by excluding all others. Male substances can be broken down within the female and used for egg development. Male-derived proteins are likely to be present in the female fat body, follicle cells, and oocytes and used for her reproduction.

The evolution of insect mating systems has been largely influenced by the asymmetrical reproductive interests of both males and females. Females tend to invest more of their resources into egg production than males do in producing sperm. They produce not only gametes but also a large package, the egg, which represents a huge outlay of metabolic assets. For the most part, males only contribute sperm to the next generation, and because the sperm are small and plentiful, there is little risk for males to invest in a single mating. They rely on the large investment of the female and allocate their resources to make themselves more successful at inseminating females than at investing in parental behavior. The tendency of females to make a greater parental investment in their offspring may also explain why the males, which usually contribute far less to their offspring, are subject to stronger sexual selection and compete more vigorously for mates. Females are thus a limiting resource that males compete for; there is considerable competition among the males of a species for the insemination of females and the subsequent transmission of their genes to ensuing generations. A strong selective pressure therefore exists for males to produce substances that give them a reproductive advantage by preventing a female from subsequently mating with other males. Lepidopteran males that produce a larger spermatophore are able to delay the subsequent mating of the female by providing a greater activation of stretch receptors in the female genital tract. However, as the male contributions and his parental investment increase with nuptial gifts or costly ejaculates, there can be a reversal of roles. At high densities and poor nutrition, female Mormon crickets undergo a role reversal and compete for access to sexually receptive males, which may reject certain females based on their smaller size.

Most male insects are **polygynous**, mating with more than one female during their lifetimes. Sexual selection favors males that mate with many females, because they can fertilize more eggs. Although a female cannot produce more offspring than the number of eggs she develops, males can increase their fitness by mating with many different mates. Monogamy in males is rare, and may be best exemplified by the honey bee drone that dies after detaching his genitalia and leaving them inserted in the female's genital tract. In contrast, most female insects, even though they produce a relatively small number of eggs, still must make a large investment in their production and the survival of offspring. It may be in the best interests of the female to mate with more than one male during their lifetimes and use the genetic products of several males.

Female insects can engage in several different patterns of receptivity. Females may either mate only once in their lifetimes or mate multiply. Most solitary bees and wasps and many dipterans mate only once. In females that mate multiply, mating may occur throughout their lives or be limited to specific periods of receptivity. An example of an insect with a defined period of receptivity is the harvester ant, *Pogonomyrmex*, which copulates with several males on the day of her nuptial flight but is afterward unreceptive. Female *Drosophila* mate repeatedly during their lives, but only at widely spaced intervals. In contrast, *Anthidium* bees will mate with any male that the females encounter at any time during their lives.

The copulatory acts and positions in insects are believed to have originated with the indirect transfer of a spermatophore, the most primitive means of transferring gametes. In apterygotes, the male deposits the spermatophore on the ground and the female acquires it independently, but the act is more intimate in the thysanuran family Machilidae where the male produces a thread leading from the spermatophore that guides the female to it. The mating position of the female when this occurs, lying above the male, is considered to be the most primitive, from which all other mating positions evolved. In male dipterans, the genitalia must rotate after the adults emerge to allow them to acquire the proper position for mating to occur. This rotation may be as much as 360°, curiously ending at the same point as it began.

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CHAPTER 5

Behavioral Systems

Behavior can be most simply defined as what living organisms do. Behavioral physiology examines how they do it and the physiological reasons for why a particular behavior may be expressed at a particular time. Behavior, like morphological structures, is a phenotype whose development and expression are influenced by both genetic and environmental factors. Although usually simple to observe and requiring little in the way of instrumentation to measure, behavior represents the end result of a complex series of nervous impulses and muscular contractions that are initiated in a specific order and that ultimately result in the expression of what we see as a spatial displacement of the insect. What may be observed as a single behavior may actually consist of a chain of individual behaviors that constitute it.

Behavior is largely a function of the expression of specific genes that have been selected for when an animal has been successful at living in a particular environment. A number of human genes have been linked to behavior through various heritable disorders, but in general the genetic basis of behavior applied to humans has been controversial, either because it is believed that humans are so complex they have probably evolved to a degree where they are somehow different than other animals or that our unique consciousness allows us to override the determinism of our genes. It is virtually impossible to separate the

influence of genes from that of human culture. In insects, the expression of behavior is significantly less affected by these factors, and the evidence for a genetic basis for behavior is much more obvious. As we will see, the role of genes in the repertoire of available behaviors is relatively clear in insects and other invertebrates.

WAYS OF LOOKING AT BEHAVIOR

A common pitfall in the study of animal behavior is **anthropomorphism**, the attribution of human characteristics to nonhumans. The true mechanisms that underlie behavior are obscured when insects are assumed to have human qualities such as motivation, anger, hunger, and lust. For example, a female mosquito does not approach a host for a meal of blood because it is hungry and wants to reproduce. Its nervous system is simply responding to the environmental stimuli that are translated by sensory receptors and that activate a series of genetically programmed behaviors that have been shaped by natural selection. It is not necessary to postulate that the insect has any purpose or goal; it is only responding to the stimuli it receives with stereotyped behaviors that involve the orderly activation of muscle groups.

If the physiological basis of behavior is most simply considered as a temporal series of muscle contractions, then the units of behavior are composed of the systems of sensory receptors, nerves and muscles that control the physical displacement. The nervous system contains a number of innate prewired endogenous motor programs known as **fixed action patterns**. Generally, fixed action patterns have several common characteristics: they are fairly stereotyped and are found in all individuals of the species that displays them; they are initiated in their complete forms by certain releasers; they occur in the absence of positive feedback once they are initiated; and they involve the coordination of several different muscle groups.

An example of this stereotypical behavior is the avoidance behavior of some noctuid moths to the ultrasonic cries of bats (see Chapter 11). The moths have a tympanum on either side of the abdomen that is innervated by two sensory receptors of differing sensitivity. When the moth is far from the bat's attempts to echolocate, only the more sensitive receptor is triggered, initiating a directional response away from the area of the bat. When the moth is closer, the less sensitive receptor is additionally triggered, initiating an evasive dive by the moth instead. With only four sensory receptors, nervous connections, and the necessary muscles, the moth can execute behaviors based on fixed action patterns that are both economical in design and essential for its survival. It is not necessary to consider the moth's conscious awareness of the bat or its fear of being captured, and indeed such considerations only prevent the true mechanism of behavior from being understood. The elegance of the system goes one step further in

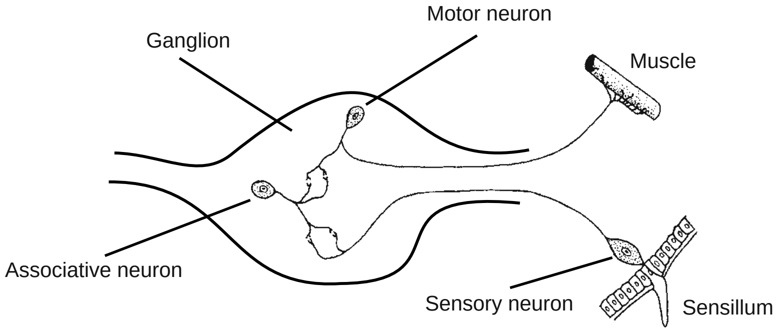

FIGURE 5.1. A reflex arc, with an associative neuron in the ganglion connecting a sensory neuron with a motor neuron.

some pyralid moths, where ultrasonic hearing is used not only for bat evasion but also for mate attraction. Male moths, using the tymbals at the base of their forewings, produce a different ultrasonic signal than bats employ, stimulating the females to approach them for mating. Two different signals received by the same sensory receptor in females result in adaptive behaviors that are quite different.

The simplest innate behavior and the most primitive of the fixed action patterns is the **reflex**. In a reflex arc, the dendrites of a sensory neuron synapse with an associative neuron that then synapses with a motor neuron that innervates a muscle (Figure 5.1). Stimulation of the receptor causes an immediate contraction of the muscle. More complex fixed action patterns include **kineses** and **taxes**. Kineses are locomotor responses to stimuli that are nondirectional. The intensity of the response may vary with the strength of the stimulus but is not related to its direction. With a photokinesis, an insect might respond to light by becoming more active and moving without any particular direction whenever the light is perceived. This would ultimately cause the insect to be displaced from areas of light and settle in dark areas where it is not stimulated to move. In contrast, taxes are directional movements toward or away from a stimulus. For example, a negative phototaxis would cause an insect to move away from the source of light. In any event, a fixed action pattern is performed in response to a specific stimulus called a **releaser** when the internal physiological state puts the insect in a condition of readiness to respond.

GENETIC BASIS OF INSECT BEHAVIOR

One of the best examples of how single genes affect behavior is seen in *Drosophila melanogaster*, where specific behaviors are programmed by genes that directly regulate the development and function of the central nervous system. Mating in

Drosophila involves a specific sequence of fixed action patterns that the male must execute before copulation occurs. Mature males court females but never other males, whereas the females fail to court at all. The male first orients toward the female, standing about 0.2 mm away while facing her. If she moves, he follows her and begins to tap her abdomen with a foreleg. Next, he opens one wing and vibrates it to generate a courtship song. If the female is receptive at this point, he licks the female's genitalia, grabs her wings as he mounts her, and copulates. Females are generally more passive but are able to either accept or reject the advances of a male based on their own physiological state. Receptive females open the vaginal plate to allow copulation to take place and raise their wings so the male can grasp them. Unreceptive females may extrude their ovipositor to prevent the male from copulating.

A key gene that regulates this mating behavior is *fruitless* (*fru*). The gene plays a critical role in establishing the neural circuitry that governs these male-specific courtship behaviors. Alternative splicing, which occurs differently in males and females, generates a complex set of sex-specific zinc finger proteins. The male-specific splicing of *fru*, resulting in Fru^m proteins, is required for the expression of male courtship behavior. Fru^m is specifically expressed in neurons of the male nervous system that are involved in male courtship behavior, including the olfactory neurons that respond to female sex pheromones. Fru^m is absent from the nervous system of females, but when it does occur, they not only lose their female reproductive behaviors but actually court other males.

Some *fru* mutations affect the formation of a male-specific abdominal muscle, the muscle of Lawrence, which is essential for proper mating behavior by the male. Other alleles of *fru* block the male from displaying a courtship song. Males with mutations in the *dissatisfaction* (*dsf*) locus actively court and attempt to copulate with both males and females but have difficulty doing so because of an inability to bend their abdomens properly. Unmated *dsf* females tend to resist the courtship of males.

Rather than directly influencing behavior, another gene, *doublesex* (*dsx*) directs the morphological development of characteristic male or female anatomical structures (Chapter 4). The *doublesex* gene encodes a transcription factor that is differentially spliced into male or female isoforms and directs the development of the proper sexual structures. Males that lack the proper doublesex proteins still court females, although their male reproductive structures are absent. Whereas *fru* regulates sexual behavior, *dsx* regulates the development of the accompanying anatomical features. Both morphology and behavior are guided by analogous genetic principles.

Alleles at the *period* (*per*) locus affect the *Drosophila* courtship song, as well as the circadian rhythms of the male. Males with a normal *per* gene generate a song that makes the female more receptive to their advances, but males bearing mutant *per* alleles produce songs that are less effective in generating the necessary female receptivity. This male song is also species-specific; *D. stimulans* males produce a

song that differs from that of *D. melanogaster* in the intervals between song pulses. The *per* genes in the two species are the same with the exception of differences in their center regions. In an elegant example of how a single gene can be responsible for specific fixed action patterns, a hybrid *per* gene containing the center region of the *stimulans* gene was constructed and inserted into the *melanogaster* genome. The transformed male *melanogaster* sung the *stimulans* song instead of its own. Other genes such as *dissonance*, *croaker*, and *cacophony* are also known to alter the male song pattern.

Several other genes affect copulation, the last step in mating. Normal copulation may last between 10 to 20 min in typical *Drosophila*, but males with the mutations *coitus interuptus* or *fickle* terminate copulation prematurely. The male mutants *stuck* and *lingerer* copulate normally, but they have difficulty withdrawing their genitalia from the female. These males may even die with their genitalia still attached to the female. The fixed action patterns involved in mating have been linked to specific neurons and the genes that control their development within the central nervous system of *Drosophila*.

There is a strong genetic basis to the feeding-related foraging behaviors of insects. Some *Drosophila* larvae are characterized as "sitters" that do not move very far during feeding. Others are "rovers" that move in wide circles as they feed, covering as much as 5 cm every 10 min. After molting to the adult stage, rover flies also show an increased tendency to walk farther from a food source than do sitters. The behaviors are attributable to the *foraging* gene (*for*), which codes for a cyclic GMP-dependent protein kinase (PKG). Two *for* alleles are responsible for the behavioral phenotypes: for^R rovers have higher PKG activity than for^S sitters. The reduced levels of PKG in sitters may affect signaling pathways that influence the excitability of nerve cells. Each phenotype has a selective advantage under certain circumstances, with rovers performing optimally under high larval densities and patchy food distribution, and sitters favored when food is distributed evenly and larval densities are low.

The *foraging* gene is also involved, in a different way, in the age-related transition to foraging behavior in individual honey bees. Younger workers remain in the hive as nurse bees and are negatively phototactic, but at 2 to 3 weeks of age they become foragers that are attracted to light and venture outside the hive. The gene is upregulated in these older honey bee workers to produce a four-fold increase of PKG activity in the optic lobes and mushroom bodies that accompanies the expression of their foraging behavior. There are significant differences in the way bees and *Drosophila* forage and how the *for* cascade is implemented, but the role of this gene in behaviors associated with acquiring food has been largely conserved. In *Drosophila*, the behavioral phenotypes result from allelic variation in populations, but in bees, the gene is upregulated during an individual adult's life.

An ultimate outcome of foraging behavior is the ingestion of food. Although feeding is essential for all animals to survive, not all the substances an animal

encounters during foraging are necessarily good to eat. Many foods may be noxious, poisonous, or simply unfamiliar, but when insects are food-deprived, they are much more likely to ingest them. **Neuropeptide Y** (NPY) is a neuromodulator in vertebrates that is involved in feeding and regulating whether the foods with aversive tastes are ingested. A homolog of NPY in *Drosophila* is **neuropeptide F** (NPF), encoded by the *npf* gene and expressed in six neurons in the larval brain and numerous endocrine cells in the midguts of larvae and adults. Its receptor, NPFR1, is related to mammalian NPY receptors and is present in specific neurons related to feeding. The experimental overexpression of these receptors in *Drosophila* causes an increased ingestion of noxious food even in non-food-deprived insects. The NPFR1 neurons in *Drosophila* respond to both the insulin and NPF signaling pathways; nutritional stimuli relayed by insulin signaling modulates the intake of compromised food that is stimulated by NPF signaling and regulates the aversive response to noxious food based on the insect's nutritional state (Figure 5.2). Expression of the *npf* gene is also responsible for the attraction of *Drosophila* early instar larvae to food. Older larvae that cease foraging in preparation for pupariation no longer express the gene. When transgenic larvae that overexpress the gene are constructed, their feeding period is prolonged.

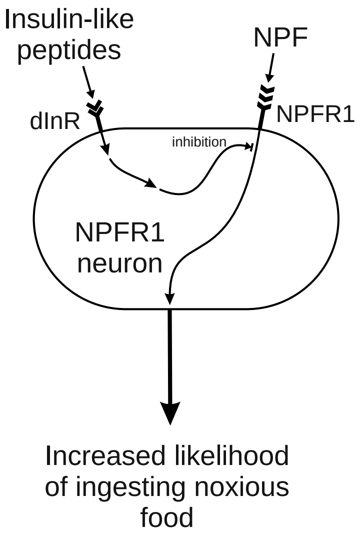

FIGURE 5.2. The control of noxious food ingestion in *Drosophila*. NPFR1 neurons integrate signals from insulin-like peptides through the dInR receptor and neuropeptide F (NPF) through the NPFR1 receptor. NPF promotes the ingestion of noxious food, while ILP negatively regulates NPFR1 activity through several steps based on the organism's nutritional state.

PHYSIOLOGY OF LEARNING AND MEMORY

Learning is a change in the behavior of an organism as a result of experience, and its ability to learn accounts for a large part of its behavior. The capacity of *Drosophila* to learn has been assessed by pairing an odor with an electric shock and then measuring the avoidance of the insect to the odor alone. The learning mutants that have been identified in *Drosophila* can be divided into structural brain mutants and conditioning mutants. Defects in brain structure account for a variety of the learning and behavioral alterations, with changes in the mushroom bodies of the brain having perhaps the most prominent role in odor conditioning. A deficit in odor learning is characteristic of several mushroom body structural mutants. For example, the *mushroom body miniature* gene (*mbm*) affects the gross anatomy of the mushroom bodies, and mutant *mbm* flies show a significant odor conditioning impairment. Conditioning mutants include *DC0*, which codes for the catalytic subunit of protein kinase A in the mushroom bodies and lessens learning ability when the mutant allele is present. A short-lived olfactory memory also resides in the projection neurons of the antennal lobe, with different odors represented by different subpopulations of these neurons.

The *dunce* (*dnc*) mutants similarly affect *Drosophila* learning. These mutants are unable to learn and have unusually poor memories. The *dnc* gene is largely expressed in the mushroom bodies of the brain and encodes the enzyme cAMP phosphodiesterase, and its various alleles differ in the degree of enzyme activity that is present. Some *dnc* mutants have up to eight times the normal levels of cAMP because the enzyme variant that is produced is unable to hydrolyze it, and the high levels interfere with learning. The *rutabaga* (*rut*) gene encodes an adenylate cyclase that is also necessary for normal learning to occur in *Drosophila*. Cyclic AMP signaling is thus required for learning to occur.

Associated with *Drosophila* learning are capacities for both short-term memory, reflected by transient changes in the transduction cascades within neurons, and long-term memory, involving alterations in gene expression that produce structural changes in the neurons that result in the changes of their synapses (Figure 5.3). Olfactory information from antennal lobes are coupled with the inputs of unconditioned stimuli via dorsal paired medial (DPM) neurons that express the *amnesiac* (*amn*) peptides and dopaminergic (DA) and octopaminergic (OA) modulatory neurons. The DPM, DA, and OA inputs to mushroom body neurons activate the adenylate cyclase product of the *rut* gene, which elevates intracellular cAMP. Cyclic AMP activates the enzyme protein kinase A (PKA), the catalytic subunit of which is encoded by the *DC0* gene. The active PKA phosphorylates several substrates to establish short-term memory, or by phosphorylating the cAMP-response element binding (CREB) protein, a nuclear transcription factor, for long-term memory based on new expression by CREB-dependent genes. The homologs of several of these genes have also been isolated from mammalian

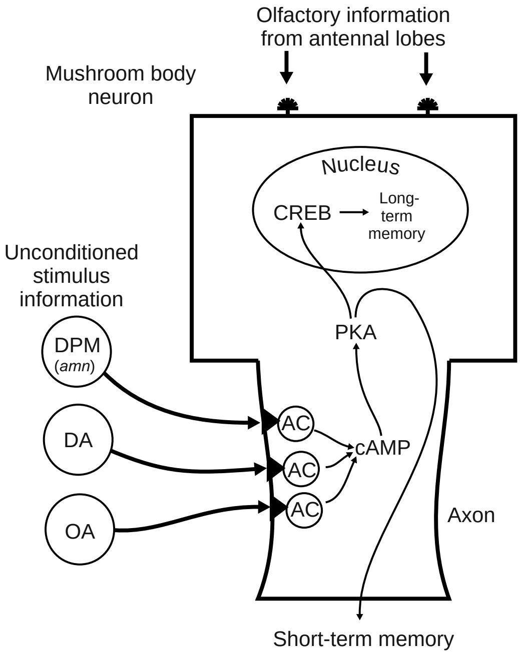

FIGURE 5.3. A model for short- and long-term memory in *Drosophila*. The antennal lobes receive olfactory information that can be associated with unconditioned stimulus information from dorsal paired medial (DPM), dopaminergic (DA), and octopaminergic (OA) neurons. DPM neurons express the *amnesiac* (*amn*) peptides. These neurons can activate adenylate cyclase (AC) that elevates cAMP, which then activates protein kinase A (PKA). PKA can establish short-term memory by phosphorylating other proteins or by phosphorylating cAMP response element binding protein (CREB) and establishing long-term memory.

systems, allowing many of these insights into *Drosophila* learning to be applied to vertebrates.

The nervous system of *Drosophila* shows a considerable degree of structural plasticity. The exposure to sensory stimuli during development is capable of altering the size and number of fibers in the mushroom bodies. Rearing flies for 4 days in constant light increases the volume of the mushroom bodies by as much as 15% compared to other flies reared in total darkness. The structure of the insect nervous system is thus capable of changing in response to environmental stimuli during development and setting the stage for the expression of different behaviors as a result of that experience.

Many insects, particularly social insects, are guided to their nests by physical landmarks. **Tinbergen**, a pioneer in the field of insect behavior, circled the entrance of a solitary wasp nest with pine cones and then moved them to a nearby location while the wasp was foraging (Figure 5.4). When the wasp

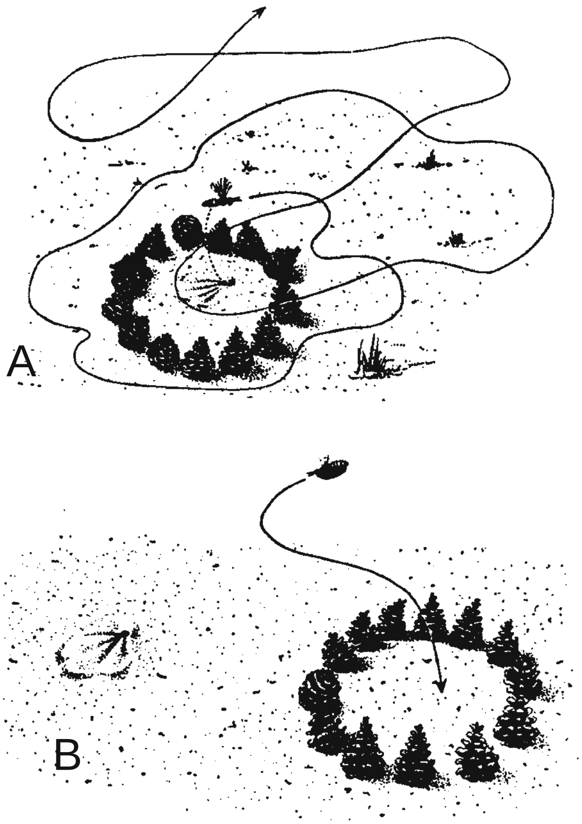

FIGURE 5.4. A. The path followed by a wasp while on an orientation flight, after its nest is circled by pinecones. B. The circle of pine cones is moved while the wasp is away foraging. When it returns, it attempts to enter the nest at the center of the cones instead of the real entrance. The wasp associates the nest opening with the pattern of cones after its orientation flight. Reprinted from Matthews, R.W., and J.R. Matthews. 1978. *Insect behavior*. Reprinted with permission from John Wiley & Sons.

returned, it searched for the nest entrance at the center of the pinecones rather than at the true entrance. This indicates that the insects are capable of recognizing and remembering environmental patterns. The mechanism of this pattern recognition is most likely **retinotopic matching**, in which retinal coordinates of a particular area are stored when the insect is at a certain position, and the insect recognizes the pattern when it matches the stored image as it returns to the same position. It is able to recognize the pattern when it falls on the same area of the retina that it was viewed with during learning. The insect must adopt a standard viewing position during both learning and recall in order to place the image in the same place on the optical receptor. The physical

storage location of these patterns may be in the mushroom bodies or optic lobes of the brain.

This pattern recognition plays a large role in the behavior of many social insects that occupy central nests with individuals that forage during the day and return to the nest to feed the brood. Although the pathways to find food may meander as the workers discover resources at great distances, the return trip is literally a beeline home rather than a retracing of the outbound route. The insect keeps track of the distance and direction it has traveled relative to the nest and is always able to return by the shortest route at the most opportune moment. A major component of this calculation is **path integration**, a mode of dead reckoning.

Desert ants in the genus *Cataglyphis* have been the favorite model for understanding this integration. They forage on hot, barren terrain that precludes the use of volatile trail pheromones or visual landmarks and must find food and return to the nest quickly or they will die under the very hot, desiccating conditions. They keep a running tally of the distance and the direction they have traveled, monitoring their forward motion and the many turns made, and summate these to compute their location relative to home to allow a quick return. The ants actually measure the distances they travel with a pedometer-type mechanism that assesses and calculates their walking distances by taking into account the number of steps taken and the length of the steps. This may be evaluated by proprioreceptors that count the actual number of steps or by the output of a walking pattern generator that produces the nervous stimuli. The navigational memory for the vector that was derived from path integration is erased when the ant arrives home so that past memories do not interfere with subsequent foraging trips. Additional information is derived from a celestial compass based on the pattern of polarized light in the sky and proprioreceptors that provide information about vertical movements with respect to gravity. In locusts that use the sun for navigation as they migrate long distances, specific neurons in the neuropil of the central complex of the brain have been identified that are sensitive to polarized light.

The nervous system of bees produces, retains, and processes information that is used to identify resources far from the nest. A worker honey bee that has identified a resource while foraging returns to the nest and communicates the location of the resource to other workers in a symbolic two-dimensional dance language that informs them about the three-dimensional location and the energy that needs to be expended to reach it. This **waggle dance**, when interpreted by workers in the hive, encodes the distance flown to reach the source and its direction relative to the sun's position. The interpretation of these dancing displays allows the observers to identify the location of resources without having direct sensory information about them. There are thus multiple spatial memories of honey bees that appear to be stored under at least three circumstances: during

orientation flights, during flights between resources in the field and the hive, and while observing dancing displays.

The foraging bee processes visual information both from landmarks and from image motion across the visual field to create a conceptual map by path integration. The visual images, and not the energy expended in reaching the food, are central to the calibration of the physiological odometer that stores the distances in memory. The retention of direction and distance are processed separately, with the outbound flight encoding distance and the inbound flight encoding direction. The choices of the flowers on which bees forage within a patch are guided by the short-term memories of their recent rewards. The sensory and cognitive capabilities of the bee's 1-mm^3 brain are astounding, able to integrate colors and polarization, shapes and patterns, movements, and olfactory cues as it flies to and from the food. These signals are then translated into the ritualized dances that communicate the information to worker bees.

Before a bee leaves the hive for the first time to forage, it circles it several times in an **orientation flight** to fix the pattern of the hive against the background image. This occurs in both young bees that leave the hive for the first time as well as older bees that resume foraging after the hive has been moved. Subsequent bee flights from the hive are direct and do not include this orientation flight unless the landscape around the hive has recently changed. A similar visual patterning occurs in *Drosophila* and depends largely on the Rutabaga enzyme, an adenylyl cyclase, coded for by the *rut* gene. Rutabaga is required in different neurons of the central complex of the brain in order for the insect to store memories of contour orientation and vertical elevation. The same enzyme was described earlier as being necessary to mediate odor discrimination learning in the neurons of the mushroom bodies. These memories are not stored in a single location in the brain but in specialized neurons throughout the brain that are each designated to perform specific functions.

The basic neural architecture responsible for memory and associative learning during honey bee foraging has been partially identified. Antennal chemoreceptors project their axons to 160 glomeruli within the antennal lobe, where they synapse with about 4500 interneurons and 1000 projection neurons. The projection neurons extend to the lateral protocerebrum, through the mushroom bodies to the lateral protocerebrum, or through the lateral protocerebrum to the calyces. The convergence sites for the olfactory conditioned stimulus and the sucrose unconditioned stimulus are the ventral unpaired median neurons. These neurons converge with the conditioned stimulus pathway at three neuropils and are important to olfactory association and learning. The antennal lobes and mushroom bodies are independent sites for the formation of olfactory memory. The neurotransmitters acting between these neurons are octopamine and the free radical gas, nitric oxide, which activates the enzyme protein kinase A in associative neurons after multiple reinforcement. Protein kinase C is produced

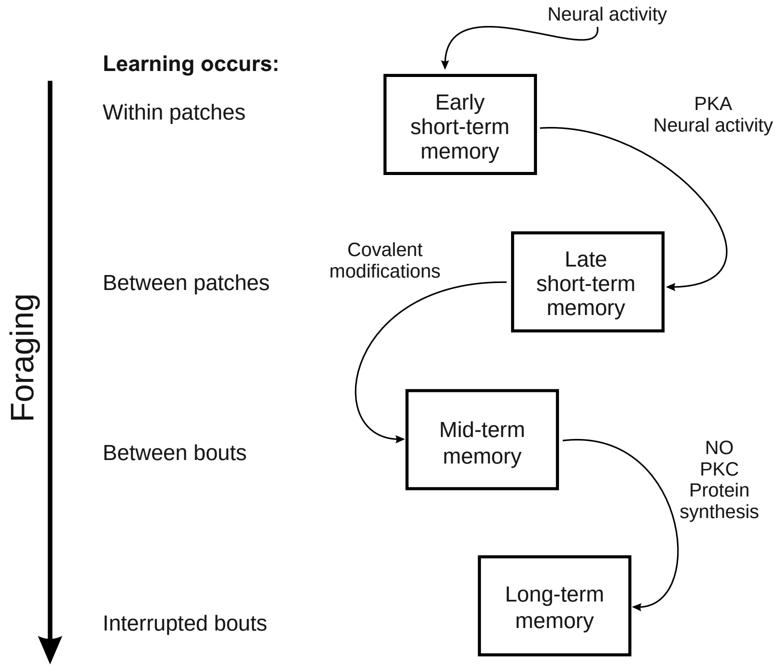

FIGURE 5.5. Memory states in the bee while foraging and their physiological correlates.

throughout the olfactory pathway and is associated with a long-term memory. These pathways and signaling molecules are associated with several different memory states in the bee (Figure 5.5).

How workers translate the dances they observe into actual foraging behavior is a mystery. In his Nobel Prize–winning hypothesis, **von Frisch** predicted that workers use the dance information to fly to the vicinity of a food source and then use olfactory and visual cues to identify its exact location. However, many studies of foraging bees have found rates of success that were surprisingly low. When foragers miss their targets, it is not because the dance information is misinterpreted or even uninterpreted, but that the visual and olfactory information in the vicinity of the food source may not be properly acquired.

HORMONAL REGULATION OF BEHAVIOR

The genes that are associated with particular behaviors may produce their effects by establishing particular neural pathways that are responsible for the manner in which stimuli are processed. The genes also code for the production of hormones that trigger the expression of the fixed action patterns that are already present

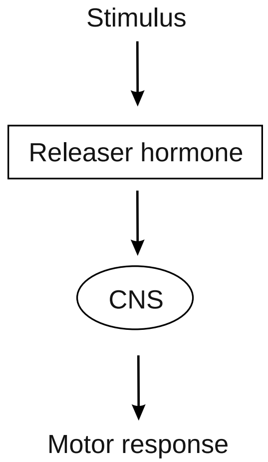

FIGURE 5.6. A releaser hormone acts on the central nervous system to produce an immediate response after a stimulus is received.

in the nervous system. These effects of hormones may be either immediate as **releasers** or occur significantly after their release as **modifiers**. In either case, they interact with the nervous system and the insect's physiological state to activate appropriate behaviors.

When hormones act as releasers, they interact with the nervous system to directly trigger a specific behavior (Figure 5.6). Given that the fixed action patterns of insects are genetically programmed, chemical messengers such as hormones, along with exogenous sensory input, are capable of releasing the behaviors that the genes have encoded. In the case of releasers, a fixed action pattern is immediately activated by the hormone's release. For example, larvae periodically display stereotyped behaviors that allow them to ecdyse from the old cuticle at the end of a molt. These behaviors are only executed when the molt occurs. The fixed action patterns involved in this complex series of behaviors are displayed after eclosion hormone and ecdysis triggering hormone are released (see *Physiology of behaviors accompanying metamorphosis*). In some mosquitoes, a releaser hormone that is produced during oogenesis inhibits the host-seeking behavior of the female until after her eggs have been laid. Another releaser hormone triggers an increased sensitivity to oviposition site stimuli to enable her to find a site to lay them. Allowing a hormone that circulates throughout the body to regulate behaviors assures that a sustained, coordinated response that involves many target systems will be mounted.

Modifier effects are subtler, altering the responsive state of the central nervous system so that a given stimulus provokes a new behavioral response in the presence of the modifier hormone (Figure 5.7). Eclosion hormone acting on adult

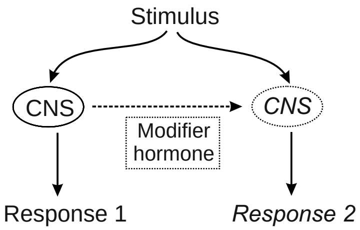

FIGURE 5.7. A modifier hormone changes the responsiveness of the central nervous system so that a given stimulus may produce a new response.

behavior is also an example of a modifier effect. A newly emerged male adult moth readily responds to the sex pheromone produced by the female, but if the moth prematurely ecloses when the pupal integument is artificially peeled off the pharate adult, the resulting adult fails to respond until after eclosion hormone is released or experimentally injected. The hormone removes a block in the central nervous system that allows the male to respond to the female sex pheromone. Eclosion hormone, acting as a modifier hormone, changes the way that the central nervous system is able to respond to a stimulus.

Aggression in vertebrates results from a depletion of serotonin, but in invertebrates the opposite is true. Increases in serotonin and octopamine are associated with increased aggressiveness in several insects including ants and crickets. Male crickets typically defend their burrows against other approaching males with a series of stereotyped aggressive behaviors, followed by a song expressed by the winning male. The loser in the interaction retreats and no longer displays aggression, but the behavior returns after a brief flight, reactivated by a central pattern generator in the thoracic ganglion. Octopamine does not initiate the aggressive behaviors but acts as a neuromodulator to enhance aggressiveness by raising the threshold for retreat from an opponent. Acting as modifiers, biogenic amines establish a level of excitability that causes an increased response to stimuli that would otherwise not elicit aggression.

A subset of modifier hormones are **organization hormones** that affect the nervous system during a critical period of development to later create permanent changes in behavior. The queen-worker castes of honey bees are determined during a developmental window, when larvae destined to be queens are fed royal jelly that causes an increase in JH that is reflected in metabolism and differences in gene expression.

Because fixed action patterns reside in the central nervous system, substances that affect nervous transmission can sometimes alter their expression. When the parasitoid wasp, *Ampulex compressa*, finds a cockroach prey, it stings it in both the subesophageal ganglion and the brain before laying an egg on it. The sting does not immediately paralyze the cockroach but instead modifies its behavior. The venom from the sting causes the cockroach to express its repertoire of

grooming behaviors, after which it becomes docile and can be led to an isolated site where oviposition then occurs. In this case, it is an injected allomone, by its proper definition, and not a hormone, that targets octopaminergic neurons and causes the fixed action patterns associated with grooming to be played out before the cockroach is no longer able to control its behavior at all. The venom also retards the metabolism of the cockroach in order to keep it alive longer and make more nutrients available to the wasp larva while it develops.

PHYSIOLOGY OF CIRCADIAN RHYTHMS

Living things evolved under daily cycles that alternated between the heat of day and the cold of night, and the ability of organisms to anticipate these cycles using internal clocks were favored by natural selection. The physiological systems of most organisms are regulated by endogenous clocks that are based on this circadian, 24-hour rhythmicity. These rhythms exist not only for behavioral systems in insects but also for tissue systems including those involved in feeding, excretion, reproduction, locomotion, and the integument, as well as for many key endocrine organs. Circadian responses evolved to coordinate the activities of organisms with the daily cycles of environmental conditions, allowing them to anticipate the fluctuation of key factors based on photoperiod and to modify their overall responses accordingly. Circadian rhythmicity is among the best example of the link between genes and behavior.

Insects were among the first animals to have their circadian clocks localized to specific areas of the brain. There are clusters of dorsal neurons, dorsal lateral neurons, and large and small ventral lateral neurons in the brain of adult *Drosophila* that express the genes *period* and *timeless* involved in the circadian feedback mechanism described next (Figure 5.8). In addition, peripheral clocks also control the local physiology of many other tissues, including sensilla in the antennae and wings, other brain neurosecretory cells and endocrine glands, the compound eyes, midgut cells, and the reproductive tissues of both sexes.

Circadian systems are made up of three components: the **circadian clock** that generates an approximately 24 hour oscillation, **input pathways** capable of sensing timing cues and entraining the clock to external signals, and **output pathways** that couple the clock to actual physiological rhythms. The major input pathway involves the flavoprotein **cryptochrome** (Cry) that is found in the nucleus of most clock neurons. Cry is believed to be the ancestral molecule that originally allowed organisms to respond to light by regulating gene expression. Its direct response to light adjusts the clock to changing light and dark cycles. Cry is light sensitive and only accumulates in the dark, so its highest levels are present in the early part of the day. The activation of Cry affects several transcription factors in the nucleus, including **timeless** (Tim), **period** (Per), **clock** (Clk), and **cycle** (Cyc) that form negative feedback loops activating

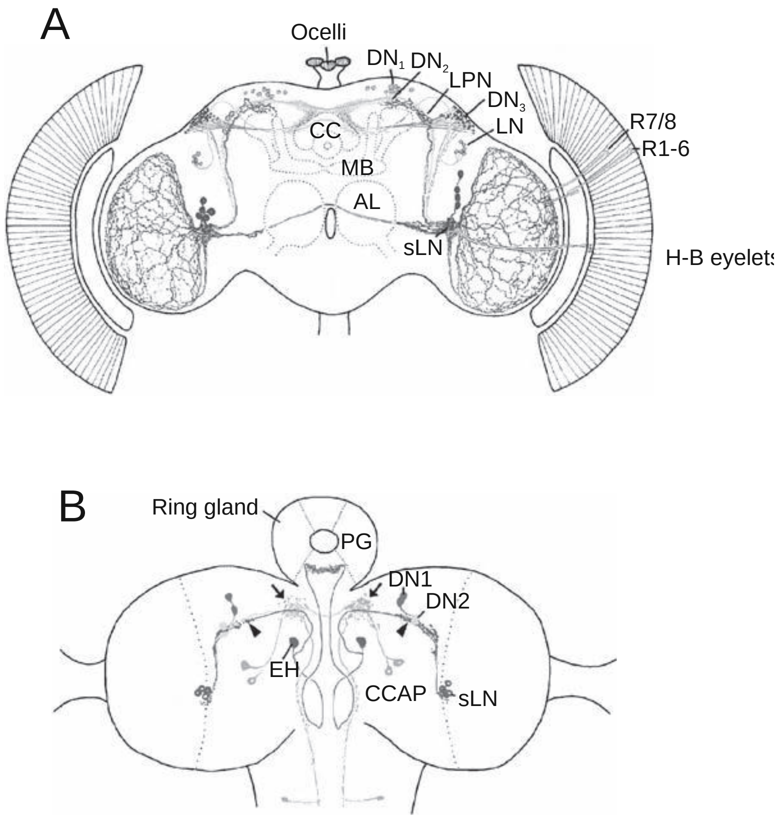

FIGURE 5.8. Neurons in the *Drosophila* adult (A) and larval (B) brains that express genes involved in circadian rhythmicity. Groups of dorsal neurons (DN), lateral posterior neurons (LPN), and large (LN) and small (sLN) ventral lateral neurons express *period* and *timeless* genes. The larval neurons that express crustacean cardioactive peptide (CCAP) and eclosion hormone (EH) are also shown. The photoreceptor cells R1-6 and R7/8 in the compound eyes and the H-B eyelets contain cryptochrome and contribute to circadian entrainment. The prothoracic gland (PG) is contained within the larval ring gland. From Helfrich-Förster (2005). Reprinted with permission.

various target genes, including those that encode their own inhibitors and serve as the clock mechanism. Within these loops, the transcription of the clock genes is regulated by feedback from their particular protein products, resulting in rhythmic gene expression. The output pathways have not been well characterized except for the 18 amino acid neuropeptide **pigment-dispersing factor** (PDF) that is expressed in pacemaker neurons in the adult brain in response to clock gene output and affects the locomotor behavior of the fly when it accumulates, serving as a mechanism to pass on the molecular clock rhythmicity to downstream genes. PDF is thus a link between the circadian clock and possible output pathways.

Cry functions as the input pathway that allows the insect to become entrained to light. When exposed to light, Cry initiates a photodegradation of Tim, and Per is degraded by the **Double-time** (Dbt) gene product, a protein kinase. This degradation prevents the formation of a Per/Tim heterodimer that is necessary for both components to be transported to the nucleus. Both Per and Tim are unstable as individual proteins but more stable as the heterodimer. Per is dependent on the interaction with Tim for protection against degradation. With continued exposure to light by midday, Tim and Per are completely degraded. This elimination of the Per/Tim heterodimer relieves the repression of the transcription factors Cyc and Clk that form complexes that bind to the *tim* and *per* genes, causing their transcription. These mRNAs move to the cytoplasm where the Per and Tim proteins are transcribed and, in the absence of Cry-mediated degradation, form the Per/Tim complexes at dusk. During the scotophase, these Per/Tim complexes are translocated to the nucleus, reaching a peak late at night, where they prevent the transcription of Cyc and Clk until the next photophase when the Per/Tim complexes break down again and the entire cycle starts once more. Light resets the clock by reducing the levels of Tim that affect this feedback loop (Figure 5.9).

Perhaps the best understood circadian system in insects is the one that regulates developmental hormones in the blood-sucking bug, *Rhodnius prolixus*. So far, there is evidence for at least four independent circadian clocks housed in the brain, prothoracic glands, and target cells of *Rhodnius*, all of which express the clock proteins Per and Tim. Two circadian clocks are located within the dorsal and lateral neurons of the brain that are in close contact with each other

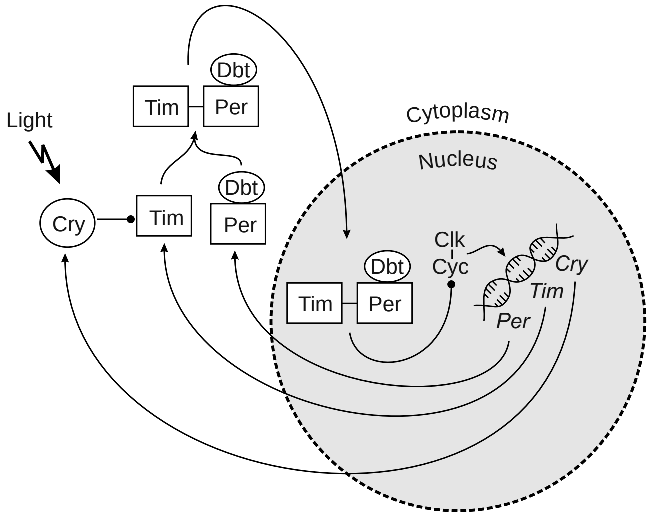

FIGURE 5.9. Components of the circadian clock. See the text for details.

and coordinate and mediate the release of PTTH from the corpus cardiacum, and a clock in the cells of the prothoracic gland establishes a rhythmicity of ecdysone synthesis. Lastly, circadian clocks present in target cells cycle the ecdysone receptor, EcR, to allow those target cells to respond to the ecdysone release only at certain times of the day. Eclosion, the emergence of adult flies from the puparium of *Drosophila*, is strongly regulated by circadian gating, allowing the emergence of the adult to occur only at certain times of the day. The clocks that modify this eclosion behavior are located both in the brain and prothoracic glands.

There are several peripheral clocks in *Drosophila* that work autonomously and are entrained collectively by the prevailing photoperiod. Their physical coupling to allow the organism to coordinate many of these outlying circadian responses is also possible. The sensitivity of *Drosophila* olfactory receptors oscillates and reaches a peak during the middle of the scotophase as a result of the rhythmicity of clock protein production in antennal chemosensilla. Circadian oscillations of clock proteins have also been identified in Malpighian tubules, cells of the alimentary tract, and the testes of moths that release sperm bundles into the vas deferens within a few hours of the scotophase.

Bees commonly use circadian clocks to enable them to predict when food would be available outside of the hive. As a result of their age polyethism (discussed later in this chapter), newly emerged worker bees are active in maintaining the hive and only begin foraging outside when they are about 3 weeks of age. The younger bees show little behavioral rhythmicity, and their expression of the *period* gene is also arrhythmic. Upon maturing into foragers, however, their production of Per and the establishment of a circadian clock correlates with their rhythmic behavior in searching for food. Bumblebees are sensitive to the duration of time intervals and can estimate the passage of time, able even to time multiple intervals simultaneously.

The circadian entrainment for many locomotor behaviors resides in the extraocular **Hofbauer-Buchner eyelets** (see Chapter 11) that can respond to light and communicate with circadian clocks in the lateral neurons of the *Drosophila* brain. These neurons are responsible for the circadian production and accumulation of the PDF neuropeptide. Flies that are engineered to produce PDF ectopically in large amounts have altered rhythms, as do mutant flies deficient in PDF production. G protein-coupled receptors for PDF are present in nonclock brain neurons in addition to clock neurons, suggesting that the peptide may also play a role in feedback on the clock mechanism. The extraocular **laminar organs** within the optic lobes are also nonvisual photoreceptors containing UV opsins that may be involved in circadian entrainment.

Temperature fluctuations and social interactions can entrain the internal clocks of insects. A temperature cycle differing by 8° to 10°C can entrain the eclosion of adult *Drosophila*, and the activity rhythms of crickets and cockroaches are altered by thermoperiod even after the cells of the circadian clock are removed

from the brain. Temperatures affect the stability of mRNA and transcription factors, altering rhythms by influencing the production of various clock components such as Per and Tim. The olfactory cues received during social communication among adult *Drosophila* that are placed in groups can reset the circadian clocks that regulate locomotor behavior. The physiological mechanism operating in these situations is not understood.

INSECT SLEEP AND AROUSAL PATTERNS

One of the most basic circadian cycles found across the animal kingdom is one of rest and activity. All animals show daily activity cycles, and the rhythmic period of inactivity in vertebrates is what we call sleep. To be considered sleep-like, an organism's inactive state should satisfy five criteria: there must be a species-specific posture when resting, a regulatory mechanism must account for the state, the behavior must be circadian in nature, there must be an increased sensory threshold for arousal, and the state must be correlated with changes in the central nervous system. Of all the insects, *Drosophila* adults have been studied the most with regard to behavior and indeed show the propensity for a sleeplike state. *Drosophila* will choose a preferred site to become immobile for up to 2 h each day when they are relatively unresponsive to external stimuli. The duration and intensity of their sleep increases with the length of the wakeful period, indicating it is under the control of a regulatory mechanism and satisfies the conditions to be considered as a primordial sleep. We have already seen that cAMP signaling to activate protein kinase A (PKA) and the cAMP response-element binding protein (CREB) are involved in insect learning, and in mammals as well as for insects, these are increased in the central nervous system during waking and decreased during inactive states. In particular, PKA expression in the mushroom bodies appears to regulate sleep patterns in *Drosophila.* Sleepiness in both humans and *Drosophila* can be identified by a biomarker encoded by the *Amylase* gene, further supporting the relevance of insect research to the study of human sleep studies.

PHYSIOLOGY OF SYNCHRONOUS BEHAVIOR

Synchronous behavior occurs when a group of organisms displays a particular behavior as a common response to the same stimulus. Most lampyrid beetles that use luminescent flashing to attract their mates do so individually, with the male flashing rhythmically and the receptive female flashing in response. However, some tropical species of these fireflies congregate in trees and display a coordinated group flashing, activating their luminescent organ approximately every 500 milliseconds with no member of the group more than 20 milliseconds out of

phase. Their synchronization depends on whether the beetles can see one another and how the nervous system measures time.

The synchrony of flashes is controlled by an internal resettable pacemaker. Rather than all fireflies flashing in response to a leader, they flash in synchrony based on a phase entrainment, in which the flash of one male resets a neighboring male's cycle. The excitation level of the stimulus receiver's pacemaker is enhanced by the external light stimulus, which soon results in synchrony within the group of clustered males.

PHYSIOLOGY OF POLYPHENISMS

Polyphenisms are discrete alternative phenotypes that arise not from an organism's genetic information but from environmental cues that are received during development. A **sequential polyphenism** occurs in holometabolous insects that undergo differing forms of larvae, pupae, and adults during their development. An **alternative polyphenism** occurs in the castes found in many social insects, where workers, soldiers, and reproductive castes can all arise from the same genotype. In both cases, environmental factors affect hormone titers that in turn trigger developmental switches that alter the pattern of gene expression (Figure 5.10).

Insect behavior reaches its highest level of complexity in the social insects as a consequence of their ability to evolve alternative polyphenisms. Their social behavior is considered to be one of the major reasons for the dominance of eusocial, or truly social, insects on the planet. The societies in which they live exist because of their capacity for communication that allows colony activities to be coordinated. In addition, social insects have a system of division of labor in which the responsibilities for the completion of different behavioral tasks are assigned to castes that are all genetically similar but physiologically distinct.

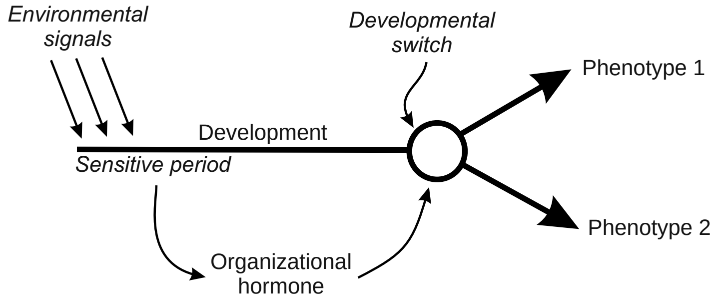

FIGURE 5.10. The control of polyphenisms by juvenile hormone. Environmental signals during a sensitive period of development trigger the release of JH later in development. The JH can switch between alternative phenotypes.

Members of the worker castes give up their ability to reproduce in favor of increased ability to defend the colony and to forage for resources. The reproductive castes have the monopoly on reproduction, and caste development is basically a matter of who is charged with reproduction in the colony.

In the hymenopterous social insects, an individual's sex is determined by haplodiploidy. Fertilized eggs produce diploid females that may be either workers or queens and unfertilized eggs produce haploid males. Fertilization is a cooperative venture between the queen and workers; the queen decides whether an egg is fertilized, but the workers construct the cells into which either fertilized or unfertilized eggs are expected to be laid. It is only during the reproductive season that the larger drone cells are constructed for the production of males. The queens and workers are all diploid females and arise from fertilized eggs, but they characteristically undergo different developmental pathways. Whether a female becomes a queen or worker is not determined genetically but is specified by the endocrine system. The passage into a particular pathway is a consequence of the endocrine milieu that is determined by the feeding program of the larva.

When worker bees feed developing diploid larvae large amounts of food and royal jelly, a mixture of their mandibular glandular secretions, the larvae develop into queens. However, if their nectar and pollen diet is more diluted and the royal jelly is fed to the same larvae for the first 2 days only, they will become workers. The diets affect the synthesis of JH by the larval CA, and larvae destined to be queens show significantly higher rates of JH synthesis. The information about the quality of the food may be transmitted to the endocrine system by the stomatogastric nervous system. High levels of JH act in at least two ways during development: they affect the proliferation of ovarian cells during the last larval instar, and they inhibit the programmed cell death that takes place in the developing gonads, resulting in increased reproductive cells in the queens. Later in the last instar, the higher titers of JH also stimulate the prothoracic glands to produce increases in ecdysteroids in queens, which direct the caste-specific protein synthesis in ovaries. The smaller amount of JH that is present in workers still gives them the potential to reproduce, but that potential is totally controlled by an existing queen in the colony. Her production of queen mandibular pheromone (QMP), largely (E)-9-oxo-2-decenoic acid, inhibits whatever limited ovarian development is possible in workers. QMP acts specifically on the endocrine system of the workers to suppress JH synthesis and affect levels of dopamine in worker brains that can change the output of neural circuits. When the queen is removed and her queen pheromone no longer circulates, a small number of eggs are able to mature into colony workers. These eggs develop into males because the workers are unable to mate and fertilize the eggs.

The number of egg-laying queens in different fire ant colonies varies based on the alleles that are present at the *Gp-9* locus. Workers in colonies where the *B* allele predominates have only one queen per colony, but workers in colonies

where the *b* allele is present accept multiple queens. *Gp-9* encodes a pheromone-binding protein, and its variants are responsible for this differential recognition of egg-laying queens by the workers that possess them. Thus, a single gene affects the regulation of a complex social interaction and ultimately the social structure of the colony.

A model of the control of the soldier-worker polyphenism in the ant, *Pheidole bicarinata*, is shown in Figure 5.11. Juvenile hormone levels, here acting as an organizational hormone, determine whether development leads to the soldier or worker caste, and these levels of JH are in turn regulated by the amount of protein in the diet. A diet low in protein causes reduced JH, and when these low levels of JH are present during a JH-sensitive period, development is channeled toward the worker phenotype. In contrast, a diet high in protein elevates JH levels, and the elevated levels during the JH-sensitive period reprogram the imaginal discs in the head so that the wide-headed soldier phenotype results. Continued production of JH in the soldiers inhibits their pupation and prolongs the larval period, causing their body proportions in general to grow larger, in addition to the increase in head size.

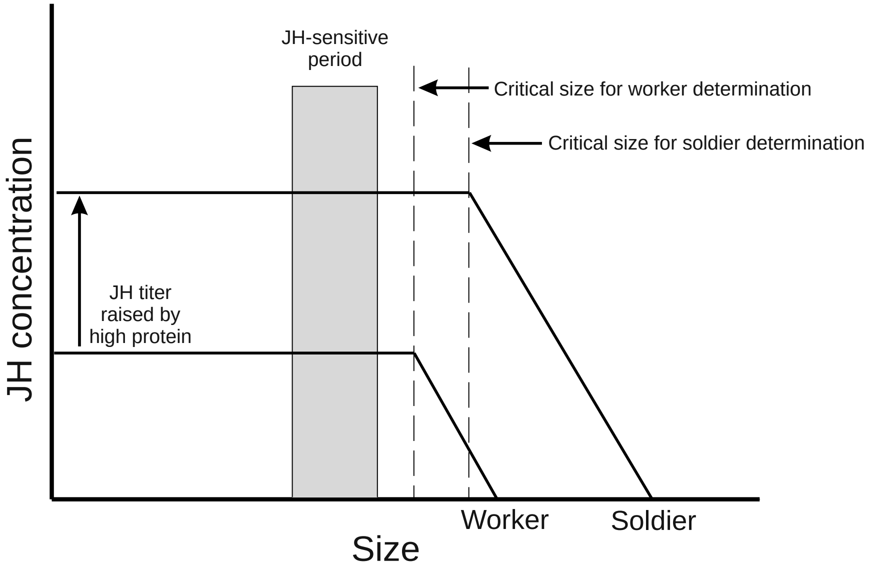

FIGURE 5.11. A model for soldier-worker polyphenism in the ant. Ingested protein raises the level of JH, and the increased JH levels during a sensitive period reprogram the imaginal discs so that development proceeds toward a soldier rather than a worker phenotype. After Nijhout (1999).

PHYSIOLOGY OF TEMPORAL POLYETHISMS

During adult life, individual honey bees show a stereotyped age-related change in the tasks they perform that is known as **temporal** or **age polyethism**. The younger honey bees act as "nurse bees" for the first 2 to 3 weeks, caring for and feeding larvae and the queen. Older bees take the roles of hive maintenance and food storage. The oldest bees, generally about 3 weeks of age until their deaths 3 weeks later, are the foragers that search for nectar and pollen and defend the hive against intruders. These behavioral changes occur in an individual bee as it ages and result from a complex interaction between the endocrine environment, the social environment, and the structural changes that occur during development.

The hypopharyngeal glands of the younger workers normally produce the larval food, but as these glands degenerate later in life, the abilities of the adults change. Without their functional glands, they are no longer able to behave as nurse bees. Other changes in behavior with age result from the restructuring of the neurons of the mushroom bodies in the protocerebrum of the brain. The older foraging bees must develop a more sophisticated behavioral repertoire to learn the location of a food source in relation to the hive and to communicate that location to other members of the colony by performing an elaborate dance language. The mushroom bodies of the protocerebrum are generally larger in social insects, suggesting they may be involved in the regulation of these more complex behaviors. They are considered to be fundamental for information processing and memory and the association of visual and olfactory stimuli. In foraging honey bees, the volume of the mushroom body neuropil increases significantly in the transition from nurse bee and correlates with increased JH titers as the bees age. JH modulates the response thresholds of the central nervous system to the stimuli that activate fixed action patterns.

Young bees feed the brood, but as they age they undergo a transition to hygienic behavior before they begin foraging. This hygienic behavior involves identifying and removing a brood infected with bacterial and fungal diseases before the diseases are disseminated throughout the hive. The ability to detect odors of a diseased brood results from the action of the biogenic amine octopamine, which reduces response thresholds of olfactory neurons. The behavioral transition to foraging also involves increases in octopamine and serotonin in the antennal lobes, and bees treated with octopamine become precocious foragers. There are also significant changes in gene expression in the brain that result in shifts in intracellular signaling, synaptic plasticity, and spatial learning. The *foraging* gene, in particular, is transcribed in foraging workers and causes increases in a cyclic GMP-dependent protein kinase in the mushroom bodies.

The behavioral progression by honey bees is affected by the social environment of the worker. Other workers, generally the older bees, produce the pheromone **10-hydroxy-2-decenoic acid** (10-HDA) from their mandibular glands. The exchange of food within the colony by workers circulates the

10-HDA that inhibits the behavioral transition to foraging in younger bees. The queen's production of QMP inhibits JH synthesis in workers and affects their behavioral development, activating the genes associated with nursing and repressing those associated with foraging. The rate at which workers make the transition to foragers thus depends on the demographic information received about the proportion of older bees in the colony and information from the queen. This mechanism maintains the proper proportion of the various behavioral types in the colony without regard to its overall size.

The behavioral transitions are reflected in the expression of numerous genes in the central nervous system. Once the entire honey bee genome was sequenced, thousands of genes were identified that were associated with the behavioral transition from workers that engage in various preforaging behaviors to those that forage outside the hive. Several of the genes have been implicated in olfactory conditioning and synapse formation, key pathways to behavioral change. The pattern of gene expression is affected by environmental, genetic, and endocrine factors, most prominently by JH. The increases in JH titers that accompany the behavioral transition in honey bees strongly suggest that JH may be involved in the up- and downregulation of many of these genes.

PHYSIOLOGY OF BEHAVIORS ACCOMPANYING METAMORPHOSIS

The immatures and adults of hemimetabolous insects look similar and have similar lifestyles. Their metamorphosis from larva to adult is not drastic, with many larval structures persisting into the adult stage. In contrast, holometabolous insects have more extreme differences between immatures and the adults, with an intervening pupal stage that allows the more pronounced transition to occur. When holometabolous insects undergo metamorphosis, the changes that take place involve more than just the modifications of the exterior structures of the integument. In addition to the morphological and ecological differences between holometabolous immature and adult insects, there are also significant behavioral differences. The structures that appear in the adult for the first time, such as antennae, wings, compound eyes, and new legs, require musculature and nervous innervations that did not exist previously. The type of food these stages ingest, the sensory receptors required to identify it, and the behavioral processes by which the food is located also differ. Changes in behavioral repertoires that accommodate the changes in lifestyle result from a reorganization of the nervous system that occurs at the same time the outer integument is being reorganized.

During the metamorphosis of the moth, *Manduca sexta*, the reorganization of the nervous system and consequent behavioral alterations are linked to endocrine changes. Hormones affect the remodeling of the nervous system, causing the

programmed death of many larval neurons, the remodeling of others, and the proliferation of new adult neurons from nests of neuroblasts. Behavior is also affected by the new sensilla that arise and the adult muscles that replace the degenerating larval muscles. Juvenile hormones and ecdysteroids control not only the molt but also the development or degeneration of all these other systems that affect behavior.

Whether a specific neuron dies or proliferates during metamorphosis depends on its response to the peaks of ecdysteroids to which it is exposed. As discussed in Chapter 1, steroid hormones bind to nuclear receptors that affect gene transcription. The receptor that binds ecdysteroids is a heterodimer consisting of the ecdysone receptor (EcR) and ultraspiracle (USP). EcR exists in at least three isoforms, EcR-A, EcR-B1, and EcR-B2, and the expression of one of these isoforms by a neuron correlates with its response to ecdysteroids. Neurons that bear the EcR-A receptor undergo maturation when exposed to ecdysteroid, whereas those characterized by a EcR-B1 receptor isoform are associated with regression and synapse loss. The fate of the specific neurons and the behaviors they encode are thus determined by the hormone receptors that are displayed during the course of their development (Figure 5.12).

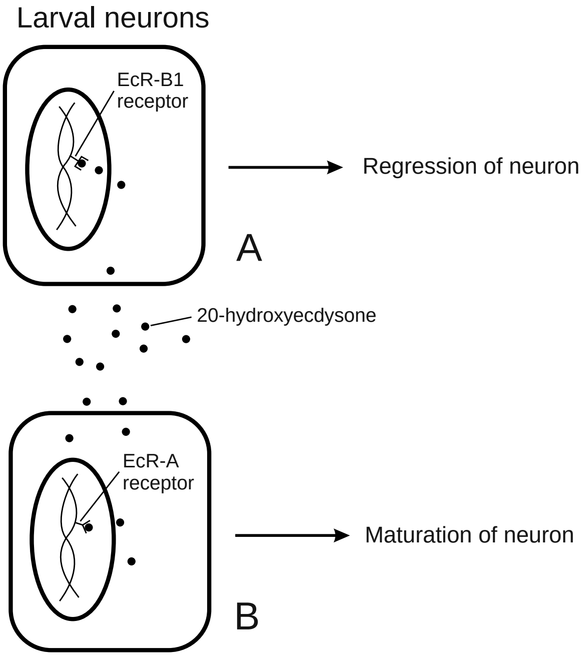

FIGURE 5.12. The fate of a neuron during metamorphosis depends on the nuclear receptors that are present. A. If the neuron develops an EcR-B1 receptor, the ecdysteroid that binds to it causes a regression of the neuron at metamorphosis. B. If a neuron develops an EcR-A receptor during its development, it undergoes maturation at metamorphosis.

The pupal stage of most insects is behaviorally passive and can be an easy mark for predators. However, one effective defense system has been incorporated into the behavioral repertoire of pupal *Manduca*. The pupae bear sharp-edged cuticular pits at the anterior margins of some of the abdominal segments that are lined with sensory hairs. These "gin-traps" are pupal-specific structures, absent in larvae and adults. Deflection of the sensillar hairs within the pits provokes an immediate contraction of the intersegmental longitudinal muscles, which draws the gin-trap under the cuticle of the next anterior segment and crushes anything that was inserted, such as the appendages of a predator (Figure 5.13). Last instar larvae do not have gin-traps, but they bear sensory neurons that innervate mechanosensory hairs that cause an overall flexion response when stimulated. During the transition to the pupa, these sensory neurons become associated with the pupal hairs that lie within the gin-trap and their axons mature in both length and the number of branches. This increased arborization of the sensory neurons occurs as the result of ecdysteroids acting in the absence of JH during the

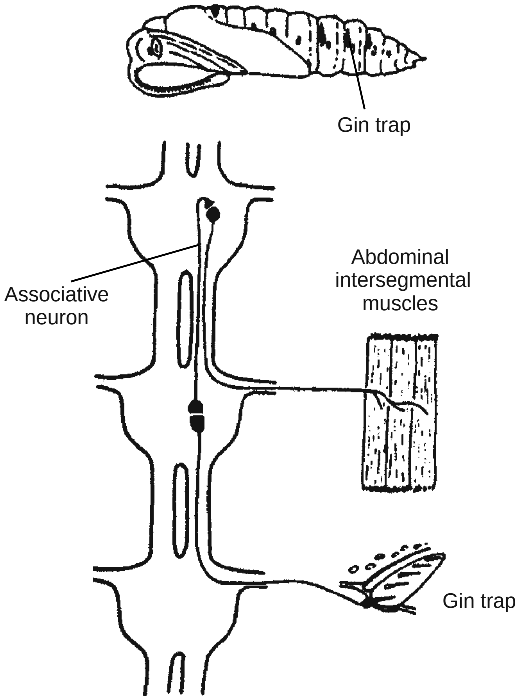

FIGURE 5.13. The mechanism of the gin trap. When the sensilla within the gin trap are stimulated, the reflex arc that is activated causes the contraction of abdominal intersegmental muscles. Reprinted from (*Brain research*, vol. 279), Levine, R.B., and J.W. Truman, 1983, Peptide activation of a simple neural circuit, pp. 335–338. Copyright 1983, with permission from Elsevier Science.

commitment peak of the last larval instar. This maturation of neurons itself is insufficient for the later operation of the gin-trap, however, because although the gin-trap and all its associated structures is fully formed in the pharate pupa, the mechanism of muscle contraction does not occur until after ecdysis. If the pharate pupa is "peeled" to remove the larval cuticle artificially before normal eclosion, the gin-trap reflex is not operative. It requires subsequent exposure to eclosion hormone in order to activate the reflex circuit so the gin-trap reflex becomes operational.

A more passive approach to predator avoidance is to hide. Just prior to their metamorphosis to the pupal stage, many holometabolous insects cease feeding and begin a period of sustained crawling until they find a suitable substrate for pupation. This stereotyped **wandering behavior** ultimately moves them to a place where they are less likely to be disturbed during the pupal period. The nervous system of a wandering larva shows characteristically sustained, intense bursts of activity in motor neurons. This wandering behavior is also induced by the commitment peak of 20-hydroxyecdysone in the absence of JH during the end of the last instar larval period. If the prothoracic glands, the source of ecdysteroids, are removed before the commitment peak, the larva does not begin wandering, but if ecdysteroids are then injected, the behavior is induced. Similarly, precocious wandering behavior can be induced if ecdysteroids are injected before the normal endogenous release occurs but after the decline of JH later in the instar.

PHYSIOLOGY OF ECLOSION BEHAVIORS

There are several stereotyped behaviors associated with ecdysis itself that are only expressed before and during the molt, including longitudinal peristaltic contractions of the body and the swallowing of air or water to cause the old cuticle to rupture. After the insect reaches the adult stage, however, because ecdysis no longer occurs, these behaviors no longer need to be expressed. There is a wave of programmed cell death in *Manduca* in which about half of the neurons of the central nervous system die within the first few days of adult life. Among these dying cells are about 50 neurons that regulate the ecdysis motor program, eliminating those cells that are responsible for outmoded or unused behaviors.

Because engaging in the molting process is wrought with danger as the immobile, defenseless insect sheds its exoskeleton, the molt is usually restricted to a specific time of the day when each species is best able to avoid predators and unfavorable environmental conditions. The timing of the molt is determined by an endogenous circadian clock that allows it to take place only during a narrow window of time. If the physiological events that are required for the molt are not completed by the onset of the window, the insect will wait for the next window to begin its molt. The system is complex, yet the stereotyped

behaviors involved have been filtered through intense selective pressures, since any errors in eclosion results in death. In crickets, 48 separate fixed action patterns have been identified that are involved with ecdysis. The various behaviors involved in eclosion are coordinated by a hormonal cascade triggered by **ecdysis-triggering hormones** (ETHs) that are released from the peripheral Inka cells of the epitracheal glands that are located on the large tracheal trunks near the spiracles. The decline in ecdysone titers following the molt initiates the complex endocrine interactions that result in the activation of these fixed action patterns.

Eclosion behavior can be divided into three phases of variable length depending on the size of the insect. In the **preecdysis phase**, lasting about 2 hours, the insect attaches itself to the substrate and begins executing movements that break the connections between its muscles and the old cuticle and begins to fill its tracheal system with air. The fixed action patterns are next activated for the shedding of the old cuticle during the **ecdysis phase**. This involves the peristaltic longitudinal contractions that enable the insect to escape from its old cuticle and may last minutes to hours. In the **postecdysis phase**, the new cuticle is stretched and hardened, again over a period of minutes to hours. Wings, if present, are inflated, and the proteins in the new cuticle are cross-linked.

The two species of silk moths, *Hyalophora cecropia* and *Antheraea pernyi*, have different eclosion gates. *Hyalophora* ecloses in the morning hours, whereas *Antheraea* ecloses just before the scotophase (Figure 5.14A). When their brains are removed surgically, the rhythmicity of eclosion is abolished in both species (Figure 5.14B). If the brains are removed and reimplanted in the abdomens, the

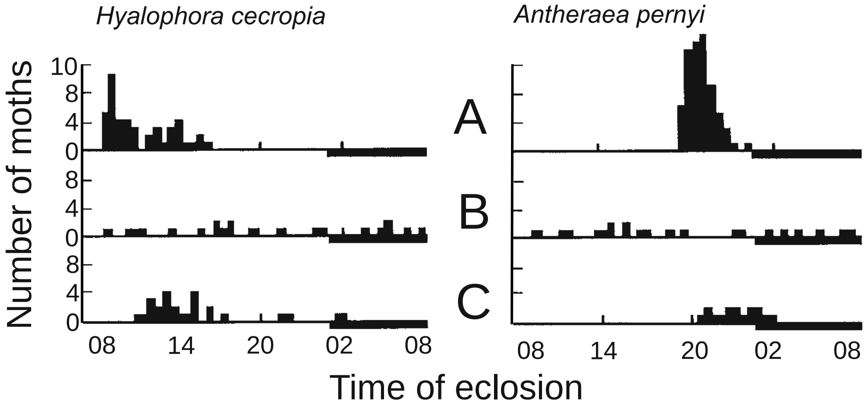

FIGURE 5.14. A. Normal adult eclosion times for *H. cecropia* and *A. pernyi*. B. Adult eclosion after the pupal brains were removed. C. Adult eclosion after the brains were removed and loose brains reimplanted. The horizontal black bar indicates the scotophase. From Truman (1973). Reprinted with permission.

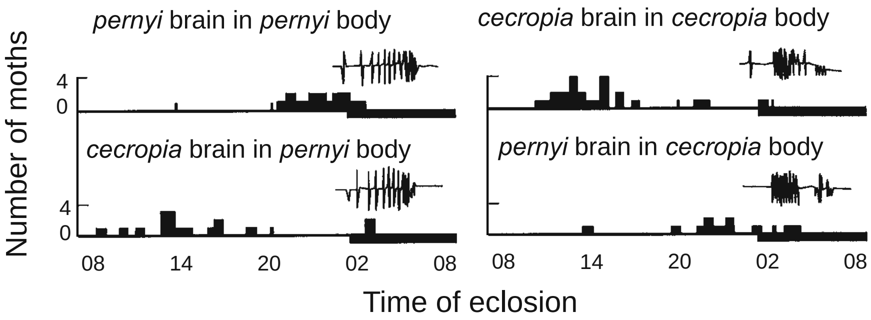

FIGURE 5.15. Effect of interchanging the brains on eclosion behavior. (*Top*) The brain implanted into the body of the same species. (*Bottom*) The brains from *H. cecropia* implanted into a *pernyi* body resulted in the *cecropia* emergence pattern, while the brains from *pernyi* implanted into a *cecropia* body resulted in the *pernyi* emergence pattern. The inset shows the traces from a lever attached to the abdominal tips lasting for approximately 2 h. The horizontal black bar indicates the scotophase. From Truman (1973). Reprinted with permission.

rhythmicity is restored, even though there are no nervous connections between the brain and the central nervous system (Figure 5.14C). Finally, if the brains are removed and implanted into individuals of the opposite species, their eclosion behavior is not only restored, but its restoration is based on the identity of the brain that is present and not the identity of the body and remainder of the central nervous system (Figure 5.15). The hormonal action is not species specific, only the timing of its release. The endocrine cascade in *Hyalophora* occurs early in the day; *Antheraea* occurs later. Either brain can activate the behavior, but only at the time it is programmed to release the hormones.

Eclosion hormone (EH) was originally thought to be central in the triggering of these behaviors because its injection activated the entire sequence of eclosion behaviors. However, studies have suggested an even greater degree of complexity involving several other hormones. Ecdysone increases before the molt and then declines, and the declining ecdysone titers are what allow the remainder of the cascade to occur and sensitize the nervous system to their presence. Synthesis of many of the peptides involved occurs with rising ecdysone titers, but release of the peptides requires declining titers. **Corazonin** (CRZR), which was initially identified as a cardioacceleratory peptide in cockroaches, is produced in lateral neurosecretory cells of the brain and released from the corpus cardiacum-corpus allatum complex and neurons in the ventral ganglia. Similarly, EH is released by neurons in the brain at ecdysis. Both stimulate the release of a number of peptide hormones from the Inka cells. Among these hormones are **preecdysis-triggering hormone** (PETH) and ETH, both of which are encoded by the *eth* gene. Like EH, injection of ETH into *Drosophila* pharate pupae results in preecdysis contractions. CRZR acting on Inka cells causes a

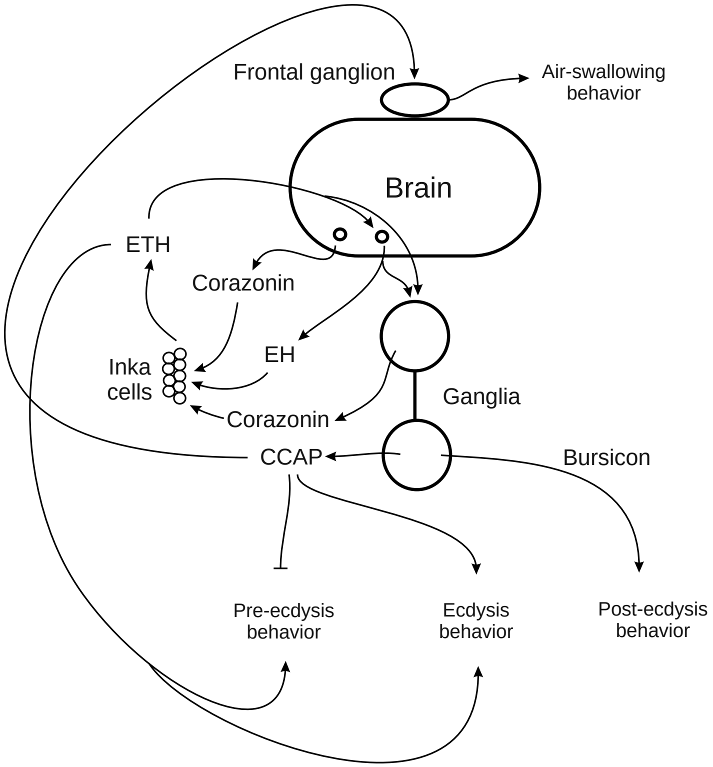

FIGURE 5.16. A model for ecdysis behavior. See the text for details. After Ewer (2005) and Truman (2005). Reprinted with permission.

low-level release of PETH and ETH, which circulates in the hemolymph and triggers preecdysis behavior. ETH also forms a positive feedback system with EH, with each stimulating the release of the other. As ETH and EH levels gradually increase, EH causes a larger release of PETH and ETH, which now mediates the transition from preecdysis to ecdysis (Figure 5.16).

Another hormone that has been implicated in eclosion behavior is **crustacean cardioactive peptide** (CCAP). CCAP was first identified as a cardioacceleratory factor in the crab and has since been found throughout the nervous systems of many insects and other arthropods. CCAP is believed to activate the motor programs for ecdysis and turn off the program for preecdysis. With both ETH and EH released together, neurons in the subesophageal ganglion and in each of the thoracic and abdominal ganglia release CCAP. When CCAP is added to a CNS preparation, the motor program for ecdysis is initiated and the one for preecdysis that was begun by ETH is terminated. This behavioral sequence is acquired and lost at every instar, assembled toward the end of each of the instars

by ecdysone signaling and subsequent gene expression. Once ecdysis takes place, the central nervous system loses its sensitivity to ETH until the next ecdysone peak. An additional target of CCAP is the frontal ganglion that innervates the muscles that dilate the foregut and cause the air-swallowing behavior to generate the internal pressure to split the old cuticle (Figure 5.16).

There are other peptides that are involved in further aspects of eclosion behavior, such as the myostimulatory product of the *hugin* gene. The hormone **bursicon** is released during postecdysis to mediate tanning and hardening. The exact relationship between all these hormones and eclosion behavior is still under consideration.

PHYSIOLOGY OF REPRODUCTIVE BEHAVIORS

Many insects do not mate until a few days after they emerge as adults. Recently emerged adult female *Aedes aegypti* mosquitoes that are approached by a male may physically couple with them but they do not retain any semen. Insemination with the retention of sperm does not occur unless the females are several days old. Juvenile hormone released within 1 to 2 days after emergence causes the maturation that allows the female to mate with successful insemination. Juvenile hormone is acting as a modifier hormone in this case, changing the way the insect responds to a given stimulus. Male *Schistocerca* locusts also require high JH titers to engage in sexual behavior.

Male mosquitoes of many species are attracted to females by their species-specific wing-beat frequency. The antennae of males have many long hairs that pick up the vibrations from female wings and activate those behaviors that are associated with mating. The antennal hairs of some mosquito species are fully erect at all times, allowing the males to receive the vibrational stimuli from females at any time of day. In other species, the hairs lie parallel to the antennal shaft during most of the day and only become erect at certain times when mating occurs (Figure 5.17). The antennal hairs must be unfolded for the males to

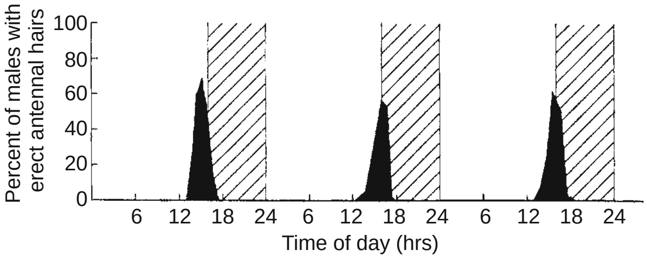

FIGURE 5.17. The rhythmicity of antennal hair erection in anopheline mosquitoes. Vertical bars indicate the scotophase. From Nijhout (1977). Reprinted with permission.

respond to female stimuli. In those species where mating occurs only at certain times of the day, usually at dusk, males with erect antennal hairs form swarms above markers and the females fly into them. The hairs allow the males to distinguish the wing-beat of those females in flight. The antennal hairs become erect as the result of the hydration of an annulus at their base. As the annulus swells, the attached hair is moved more perpendicular to the shaft, and the male is thus capable of perceiving vibrational stimuli and responding to it (Figure 5.18).

Some female mosquitoes do not simply generate the wing-beat frequency that initiates the male's response for mating, but they also actively participate in the interaction. Female *Toxorhynchites* mosquitoes receive the auditory information from conspecific males and adjust their own wing-beat frequency so the two become synchronized. This auditory interaction relies on the feedback between the sound input from the male and the motor output of female flight muscles.

The evolution of paternal care in some nonsocial insects has contributed greatly to their reproductive potential. Although females typically provide the largest investment in eggs before oviposition, once the eggs are laid and offspring hatch, both males and females may provide additional care to assure their reproductive success. Earwigs engage in maternal care, which includes constructing a burrow, guarding and grooming eggs, and protecting the eggs. An increase in JH titers is necessary for egg development, but once oviposition occurs, earwigs

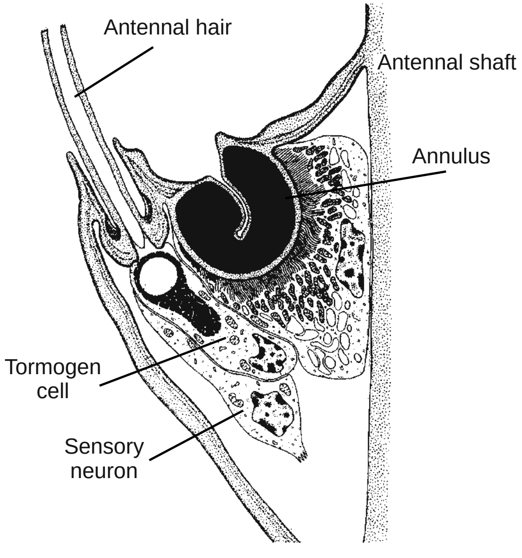

FIGURE 5.18. The mechanism of antennal hair erection in anopheline mosquitoes. The annulus swells as a result of the change in pH, causing the hair to move outwards. From Nijhout and Sheffield. (1979). Reprinted with permission.

require the JH to decline in order to express maternal care. At the time when the CA is inactive, neurohormones synthesized in the pars intercerebralis inhibit cannibalism by these normally predacious females. When burying beetles in the genus *Nicrophorus* encounter a small vertebrate carcass as a resource for their offspring, JH levels immediately increase and egg development commences. JH declines after oviposition but then rises again with the onset of the nesting phase, during which females feed the developing larvae like fledging birds with masticated portions of the carcass. JH is the only hormone that has been critically examined with regard to parental care in insects, and there are undoubtedly other endocrine factors yet to be discovered that contribute to the expression of this complex behavior.

PHYSIOLOGY OF BEHAVIORAL MODULATION BY PARASITES

Parasites that take up residence within an animal can significantly change its behavior. These behavioral alterations of the hosts are ultimately mechanisms that enhance the parasites' transmission potential and allow them to continue their life cycles.

The ciliated protozoan, *Lambornella clarki*, infects larval treehole mosquitoes in their rearing water. When the protozoan infection persists to the adults, the female mosquito's ovaries become the site of proliferation. Behavioral alteration of the mosquito host favoring the parasite involves a reduction in the host-seeking behavior of the females, as the ciliates cannot be transmitted during blood-feeding on a vertebrate host. It also involves a behavioral shift in which the female mosquito, with ovaries containing ciliates instead of its own eggs, engages in behaviors that mimic oviposition, but with parasites rather than eggs being laid on the water. The behavioral alteration of the infected mosquito host thus causes it to return to water for dissemination of the ciliate and not its own eggs. Female mosquitoes normally undergo a behavioral shift when mature eggs are present that is triggered by a humoral factor, but it is not known if *Lambonella* hijacks the mosquitoes' normal behavioral mechanism or induces its own unique behavioral switch.

A similar mechanism of false oviposition in blackflies is controlled by a fungal pathogen that commandeers the flies for dispersion. The fungus, which has yet to be identified to species, infects the early instar larval simuliid host and ultimately destroys the ovaries of the adult without it affecting the insect's longevity. The ovarian tissue is replaced by the fungus, which produces large numbers of spores that the female releases when it oviposits. After oviposition, infected flies no longer seek a blood meal.

Fever often accompanies the infection of mammals with parasites, with the elevated body temperatures aiding the hosts in overcoming the infection.

Although unable to raise their body temperatures metabolically, some insects can engage in a febrile response by moving into warmer areas, known as a **behavioral fever**. Cockroaches injected with *E. coli* bacteria prefer temperatures higher than controls. Grasshoppers and crickets infected with parasites survived better when allowed to choose higher temperatures, although the increased temperatures often resulted in poorer survival and decreased fecundity for uninfected controls. Although the occurrence of behavioral fever after infection is widespread among arthropods, it is not often clear if the host response benefits the parasite and its transmission cycle, or the host in ridding itself of the infection.

The salivary glands of mosquitoes infected with *Plasmodium* sporozoites are impaired in their production of an apyrase, an enzyme that hydrolyzes ATP and ADP to AMP and prevents platelet aggregation. This reduction in apyrase makes blood feeding more difficult and increases the probing time of the mosquito. Increased probing time results in a greater delivery of parasites and a greater number of hosts that are fed upon. Manipulating its hosts' enzyme production increases the parasites' likelihood of being distributed to more hosts.

The small liver fluke, *Dicrocoelium dendriticum*, lives in the gallbladder and bile ducts of domestic ruminants as its definitive host. The eggs of this trematode are eliminated in the feces of its hosts and are then ingested by snails, which extrude slime balls containing cercariae of the parasite. When the slime balls are next ingested by ants, the cercariae mature to metacercariae that become localized in the ants' subesophageal ganglia and affect their behavior. At lower evening temperatures, the infected ants climb to the tops of vegetation, where they remain clamped by their mandibles. Grazing ruminants do not eat ants directly but are more likely to ingest them with the vegetation when infection makes them more available and allows the life cycle of the trematode to complete.

Similar behavioral manipulations in cockroaches make it more likely for those infected with acanthocephalans to become ingested by rats. The spiny-headed worms are endoparasites of vertebrates, but require an intermediate invertebrate host to complete their life cycles. *Periplaneta* and *Blatta* cockroaches that are infected with *Moniliformis moniliformis* are less active than uninfected ones, making it easier to be caught and consumed by a rat and allow the acanthocephalan to mature in the rodent's digestive tract.

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CHAPTER 6

Metabolic Systems

All animal cells must be capable of transforming the chemical energy in the environment into electrical, mechanical, osmotic, and other forms of chemical energy in order to maintain essential life processes such as nervous transmission, muscle contraction, and the synthesis of structural components. The sources of transformed energy include the ingested food that contains complex carbohydrates, fats, and proteins that are broken down in the alimentary tract to simpler components and absorbed through the wall of the midgut into the hemolymph. The circulatory system then transports these components to all the cells of the body, which break them down further and capture the chemical energy they contain. Each cell may use the components immediately, or they may be used to synthesize reserves for later use. These processes of food breakdown, utilization, and storage are strikingly similar in insects and vertebrates, as well as in most other living things. However, some distinctive metabolic processes are found only in insects.

As is the case for most other physiological systems, the metabolic systems of only a small number of insect species have been examined, most often cockroaches, blow flies, fruit flies, or caterpillars. The evidence for the existence of complete metabolic pathways in insects has often been based on the presence of certain key enzymes, reaction end products, or intermediates that may also exist

in vertebrate systems where the cycles were more completely identified. As in vertebrates, the determination of insect metabolic pathways is complicated by the presence of symbiotic microorganisms that may provide some of the steps missing in the insect, particularly in those systems that require trace components such as vitamins. Some wood-feeding insects depend on their microorganisms to break down the lignin, and many phytophagous species can extend their possible plant hosts by relying on the bacterial detoxification of secondary plant substances. These symbiotic contributions make it difficult to establish whether the metabolic pathways are actually native to the insect system being studied.

THE INSECT ALIMENTARY TRACT

The ability of insects as a group to feed on practically every type of organic matter imaginable has been a major factor in their success, enabling them to expand into diverse ecological niches. This diversity in the food that can be ingested is reflected in the diversity of external mouthpart structures that serve as the gateway to the digestive tract. Accompanying the mouthpart diversity is a structural diversity of the insect digestive tract, with an enormous degree of specialization that varies with the particular type of diet. Because of this wide variation in insect feeding and the structures associated with feeding, what might be called a "typical" insect digestive tract does not exist. The relatively primitive gut of the cockroach, representative of an ancestral scavenger, is often used as a prototype upon which other types are described, but depending largely on the diet of the insect, there may be significant modifications to the various gut regions (Figure 6.1).

Extra-Oral Digestion

Many predaceous insects employ **extra-oral digestion** as a strategy to utilize prey that are too large to be swallowed whole. The predator typically first introduces a venom to incapacitate the prey, followed by digestive enzymes that liquefy the prey in its own exoskeleton (Figure 6.2). Both prey contents and digestive enzymes are then ingested and digestion continues in the gut. Extra-oral digestion extends the surface area of the digestive tract, actually making the prey an extension of the predator gut and allows small insects to utilize larger prey. The digestive enzymes originate in the salivary glands or the gut, consisting of proteases, carbohydrases, and lipases. Carabid beetles illustrate the advantage of using the prey exoskeleton as an enclosure for extra-oral digestion. Over 80% of prey proteins are recovered using extra-oral digestion compared to less than 50% when feeding directly on meat.

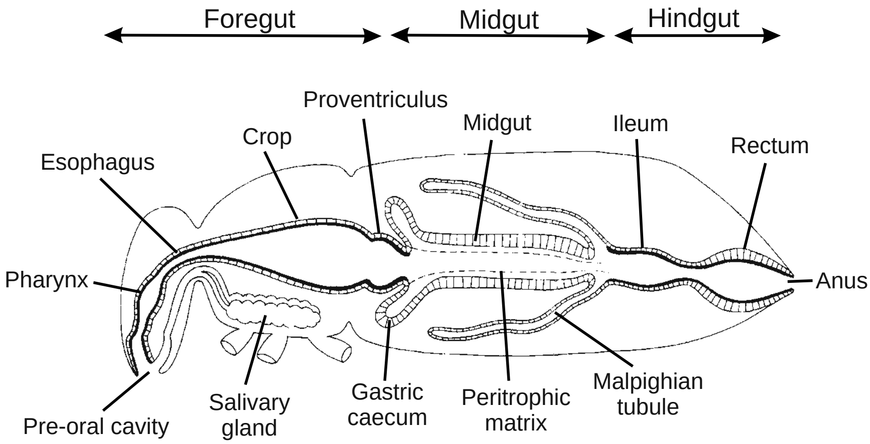


FIGURE 6.1. The three major divisions of the insect digestive tract — foregut, midgut, and hindgut — and their components. From Dow, J.A.T. 1986. *Advances in insect physiology*, vol. 19, pp. 187–328. Copyright Academic Press.

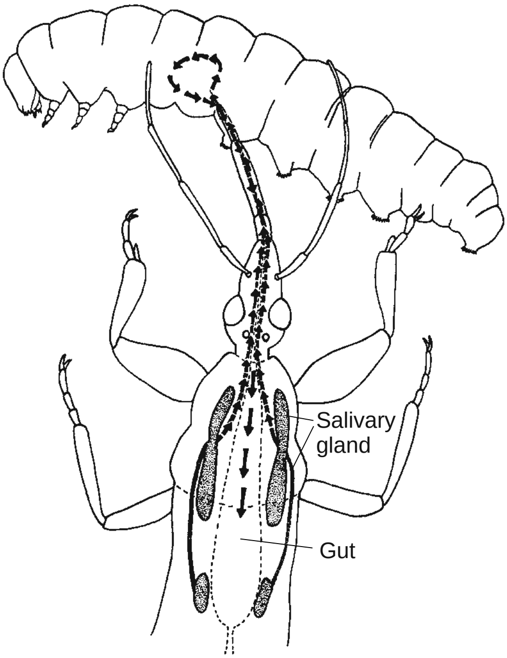


FIGURE 6.2. Extra-oral digestion, in which salivary gland components are injected into prey and then sucked into the gut. The arrows show the flow of digestive materials. From Cohen (1998). Reprinted with permission.

BASIC GUT STRUCTURE

The digestive tract consists of a tube of epithelial cells running from the mouth to the anus. It is divided into three major regions based on embryonic origins and physiological functions: the **foregut**, **midgut**, and **hindgut** (Figure 6.1). The **stomodeum** and **proctodeum** both arise as invaginations of the embryonic ectoderm and produce the foregut and hindgut, respectively (Figure 6.3). The midgut forms from endodermal tissues associated with the developing foregut and hindgut, the **midgut rudiments**, and bridges them to form the continuous gut during embryogenesis. The midgut epithelium is the only insect tissue that differentiates from endoderm. The Malpighian tubules may appear to arise from the midgut, but they actually insert between the midgut and hindgut as ectodermal evaginations of the hindgut.

The gut is supported in the body cavity by **extrinsic visceral muscles** whose contractions dilate the lumen of the gut (Figure 6.4). A number of **intrinsic visceral muscles** are also present, consisting of circular and longitudinal muscles that permit the gut to contract and undergo peristalsis. Because they are derived from ectodermal cells, the foregut and hindgut are lined with

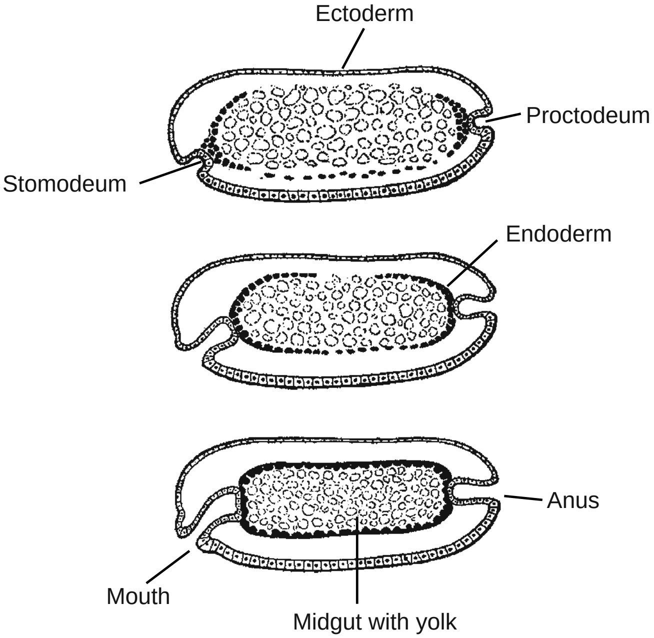


FIGURE 6.3. Embryonic derivation of the digestive tract. The hindgut and foregut are derived from embryonic ectoderm, whereas the midgut forms from endodermal tissues and connects with the blind ends of the stomodeal and proctodeal invaginations. From Snodgrass (1935).

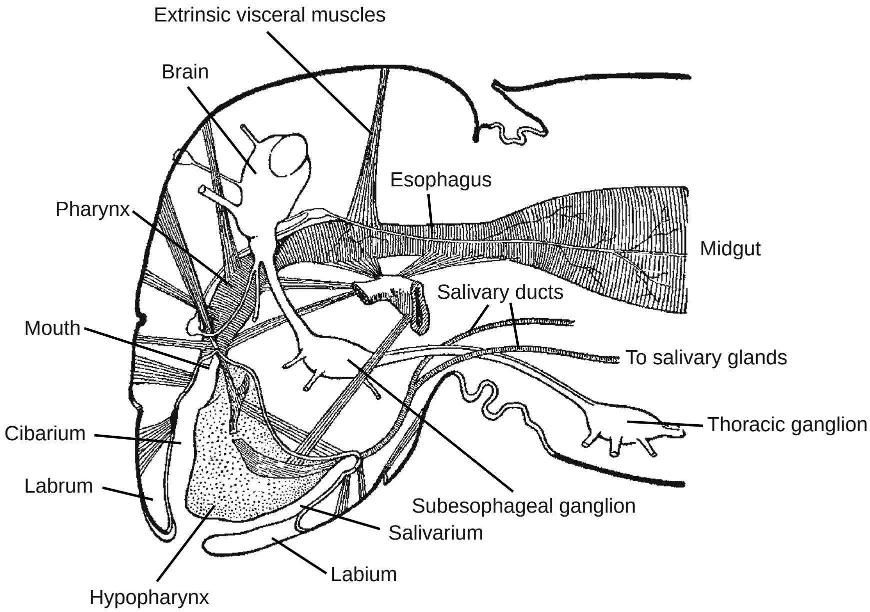


FIGURE 6.4. A section of the head showing the suspension of the digestive tract by extrinsic visceral muscles. From Snodgrass (1935). Reprinted with permission.

cuticle and molt along with the rest of the epidermis, a feature that has important implications for digestion and absorption in those regions. An important attribute of the insect digestive tract is its spatial compartmentalization, a theme that will be apparent throughout subsequent sections.

Anterior Structures and the Foregut

Primitive insect ancestors had a pair of walking appendages on each segment (Figure 6.5). Along the evolutionary path leading to insects, the appendages on the abdomen were either lost or modified into external genitalia or sensory cerci, and the appendages borne on the segments that were destined to be the head capsule were modified to manipulate food and became closely associated with the mouth. The present-day mouthparts of insects and all their variations thus originated as external appendages that ultimately surrounded the true mouth and created a **preoral cavity**. In mandibulate insects, the cavity is divided by the **hypopharynx** into an anterior **cibarium** and a posterior **salivarium** (Figure 6.4). In many mosquitoes, the cibarium is armed with cuticular spines that lyse red blood cells before they pass into the midgut. A pair of **salivary glands**, modified from the epidermal cells of the labium segment, empties their secretions

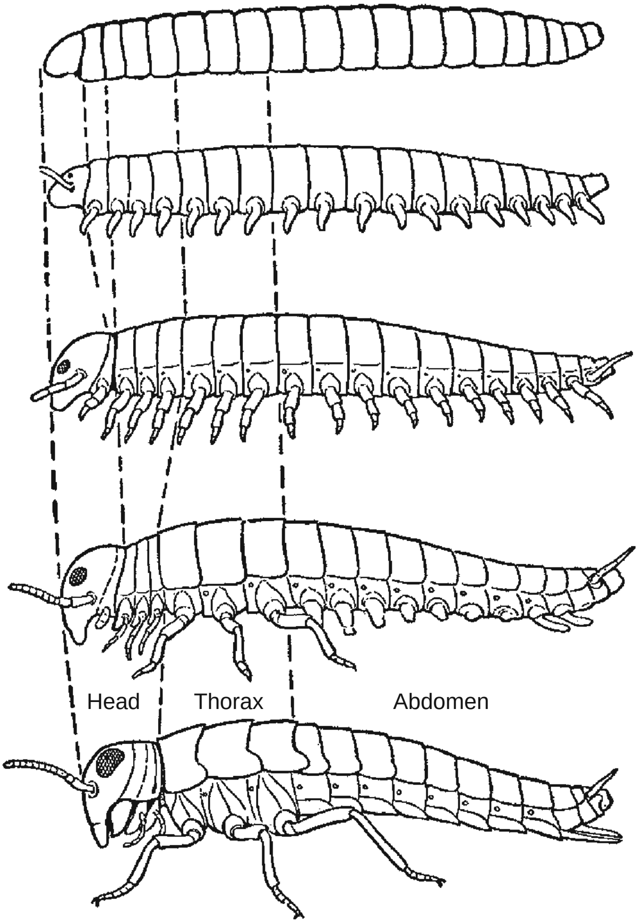


FIGURE 6.5. A probable sequence in the evolution of insects from a primitive annelid ancestor. The appendages on all body segments were reduced and segments were consolidated into functional groupings. From Snodgrass (1935). Reprinted with permission.

into the salivarium through the cuticle-lined **salivary duct**. In silk-producing lepidopterans, the labial glands produce the silk, and saliva is produced by the mandibular glands instead.

Insect salivary glands are either **tubular** in structure, as in many dipterans, or **acinar**, as in cockroaches. In the American cockroach the paired salivary glands consist of several lobes of grapelike secretory acini connected by ductules that fuse into a pair of salivary ducts that ultimately unite in a single salivary duct. Reservoir ducts also fuse to form a main reservoir duct that opens into the hypopharynx. The acini consist of three cells types: a pair of **peripheral cells**,

eight **central cells**, and **centroacinar cells** that are arranged between the central cells (Figure 6.6). The peripheral cells transport electrolytes and water, whereas the central cells synthesize the proteins of the saliva. The centroacinar cells produce an intima that lines the lumen. The glands are innervated by both dopaminergic and serotonergic neurons from the subesophageal ganglion and the stomatogastric nervous system.

Saliva lubricates the mouthparts as they move against each other and acts as a solvent for food. It may contain digestive enzymes that predigest the food before it is internalized and further acted upon by gut enzymes. These enzymes commonly consist of an **amylase** that breaks down starch to simple sugars and

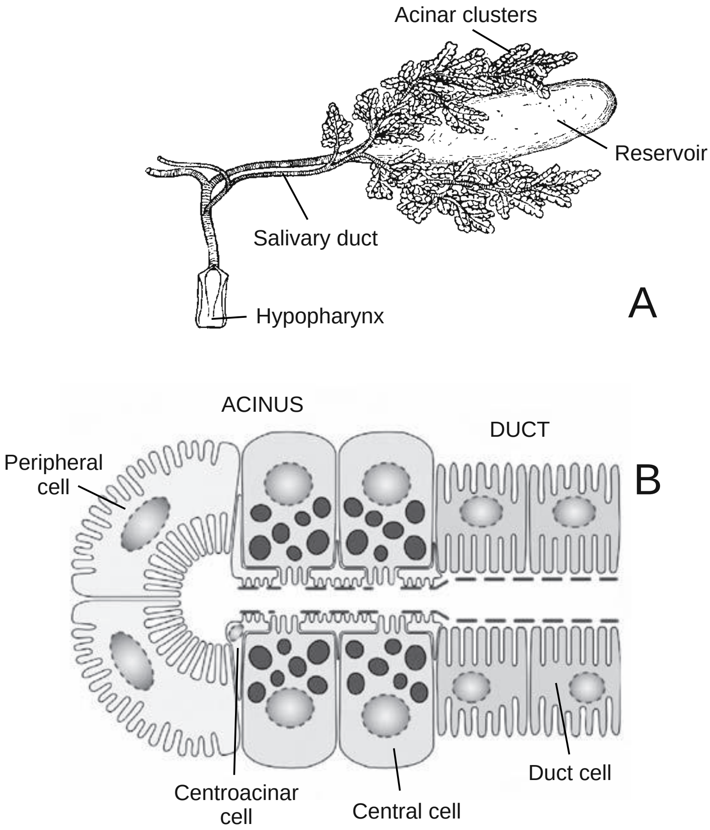


FIGURE 6.6. The salivary gland of the cockroach, *Periplaneta americana*. A. The paired salivary glands consist of grapelike clusters of acinar tissue. Emerging ductules fuse into a single salivary duct for each gland and supply a large reservoir. B. A single acinus with two peripheral cells, eight central cells, numerous centroacinar cells, and the duct cells. From Walz et al. (2006). Reprinted with permission.

an **invertase** that converts the disaccharide sucrose in the food to glucose and fructose. Carnivorous insects may produce salivary proteases or chitinases. In blood-feeding insects, the saliva contain anticoagulants and other pharmacological substances that enhance blood-feeding ability. The saliva of some plant-feeding hemipterans hardens into a **stylet sheath** that forms around the mouthparts and prevents the leakage of plant liquids when the insects feed.

The true **mouth** lies just beyond the preoral cavity and is the opening of the digestive tract. The **pharynx** is the first region of the foregut and may be modified with dilator muscles in sucking insects that expand its lumen and create a partial vacuum to take up fluids (Figure 6.4). The **esophagus** comprises the region of the foregut just beyond the pharynx and is usually a simple tube that leads to the midgut, but it may be modified into a distensible **crop** that is used to store food. In adult Diptera and Lepidoptera, the crop is a diverticulum that is separated from the rest of the esophagus by a short duct fitted with a valve (Figure 6.7). Pharyngeal receptors can determine whether ingested food enters the diverticulum or the crop by activating the valves that shunt the food. Sugar meals are first stored in the diverticulum and passed slowly to the midgut, whereas protein is sent directly to the midgut. The cuticular lining of the crop limits its absorptive capacity, and it therefore functions mainly as a food reservoir, but it is permeable to some ingested fats. Although absorption may be limited, digestion may still occur here as salivary enzymes act upon the food. A large foregut is often characteristic of insect predators that may find it more difficult to locate their animal prey and gorge themselves on infrequent large meals, storing the meal for digestion away from the host.

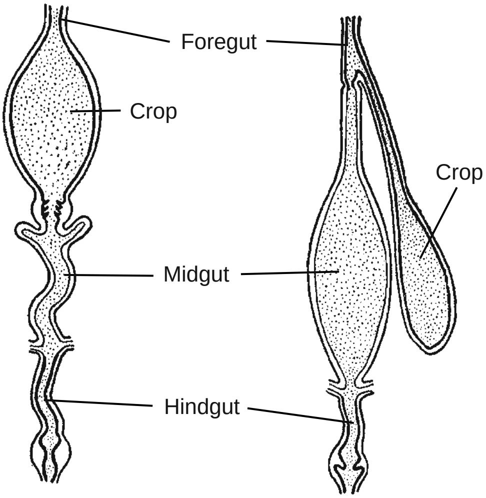


FIGURE 6.7. (*Left*) The generalized insect digestive tract. (*Right*) The evolution of the crop into a diverticulum. From Wigglesworth (1965). Reprinted with permission.

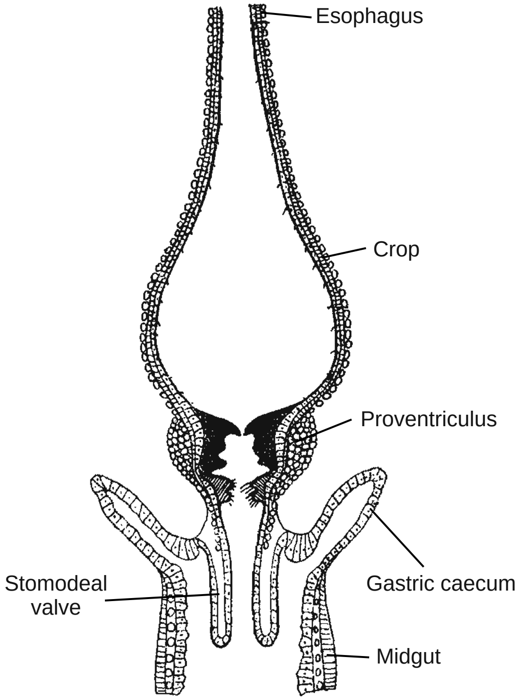


FIGURE 6.8. The location of the proventriculus between the crop and midgut. The proventriculus is modified here as a gizzard for grinding food. From Wigglesworth (1965). Reprinted with permission.

The posterior part of the foregut is variously modified into a muscular **proventriculus**. At its simplest, as in some beetles, the proventriculus is a muscular sphincter that regulates the passage of food into the midgut. In cockroaches, it is present as a gizzard lined with teeth that grinds the food before its entry into the midgut (Figure 6.8). Fleas have no crop, but a proventriculus that is lined with backwardly pointing spines breaks up the ingested blood cells before passing them into the midgut. In bees, the proventriculus projects into the crop, and armed with short spines, the structure retains nectar in the crop while passing the grains of pollen into the midgut.

Midgut

The midgut contains at least four cell types in a single epithelial layer that include **columnar cells**, **regenerative cells**, **goblet cells**, and **endocrine cells**. All cells of the midgut are derived from endodermal tissue and lack the cuticular

lining that is present in the foregut and that interferes with absorption there. The columnar, or **principal cells**, are most numerous, with borders facing the lumen that contain abundant microvilli and numerous folds, increasing the surface area for both absorption and secretion (Figure 6.9). Most nutrients in the gut lumen are absorbed through these columnar cells. They contain extensive networks of endoplasmic reticulum that are necessary for the production of the digestive enzymes. The columnar cells have limited life spans, and new ones are continually being formed from the regenerative stem cells present in groups called **nidi** that may be present as papillae on the hemolymph side of the midgut (Figure 6.10). A nidus is a stem cell niche, similar to those in the ovary and testis, whose cells divide asymmetrically to give rise to columnar and endocrine cells.

The **goblet cells** that are scattered throughout the midgut epithelium transport potassium from the hemolymph into the lumen (Figure 6.11). This movement of ions may be important for the flow of water in the gut that is necessary for nutrients to be absorbed. The gradient of potassium is utilized to drive nutrient uptake in the columnar cells through potassium/amino acid symporters at the apical regions of the cells from the hemolymph into the gut lumen.

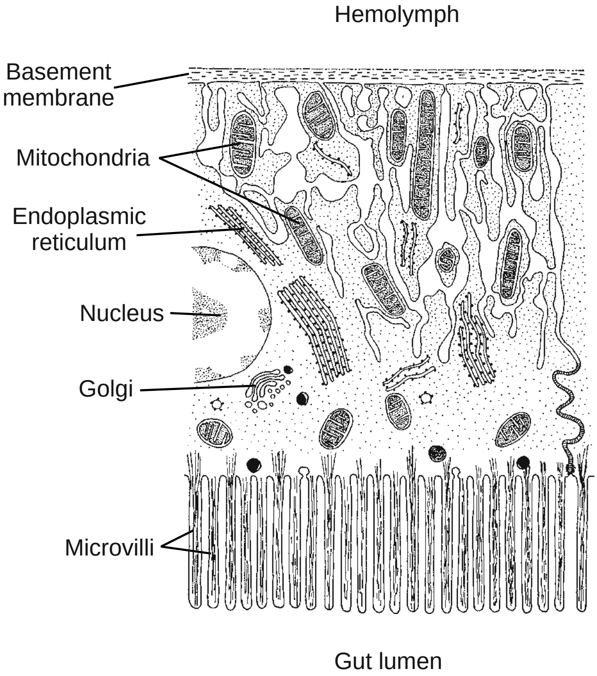


FIGURE 6.9. A typical columnar midgut cell. From Berridge (1969).

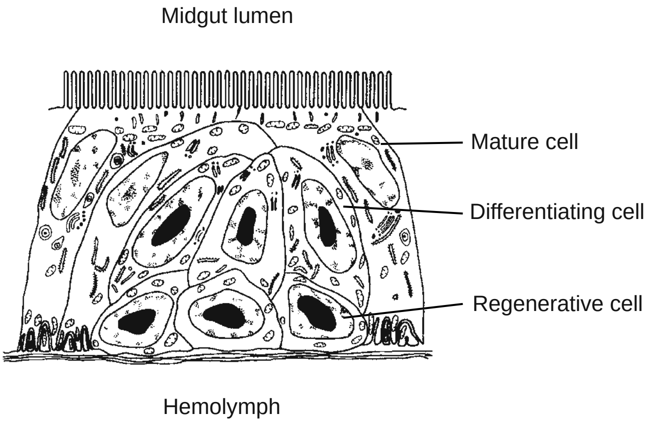


FIGURE 6.10. A group of nidi, the regenerative cells of the midgut. From Chapman (1985).

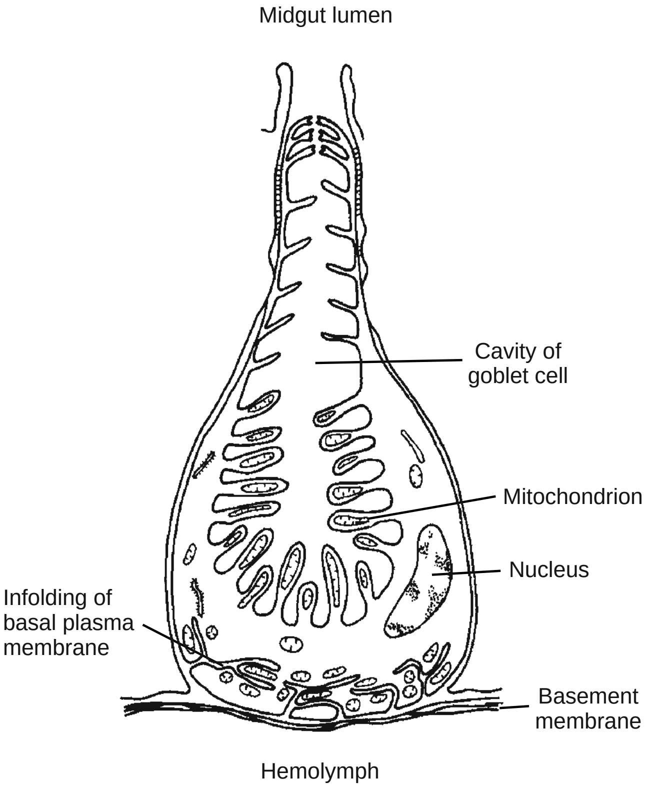


FIGURE 6.11. A midgut goblet cell. From Chapman (1985).

Plant-feeding insects, which have much higher levels of hemolymph potassium, have a reduced requirement for the driving force to move it.

In addition to conventional columnar and goblet cells, a number of endocrine cells are dispersed throughout the midgut, usually occurring as single cells but sometimes occurring in small groups. They are in contact with both the basal membrane and the lumen and contain numerous cytoplasmic secretory granules. They may represent a way for the insect to integrate its digestive and endocrine systems, assessing food in the midgut and transmitting the information to other cells by endocrine pathways. Antibodies against a large number of mammalian hormones react to the contents of these cells, including members of the insulin family, glucagons, somatostatin, β-endorphins, members of the tachykinin family, which are myotropins that may stimulate muscle contraction or act as cardioaccelerators, FMRFamide-immunoreactive peptides that may function in digestion, and allatostatin-like peptides that may regulate CA activity or have some other presently unknown functions. The physiological roles of these gut neuropeptides are not well understood.

The anterior region of the midgut may contain diverticula called **gastric caeca**. The caeca increase the surface area of the midgut for secretion and absorption and create a countercurrent flow within the gut as a result of their differential absorption of water (Figure 6.12). As water is secreted into the lumen by the posterior midgut, it moves forward to be resorbed in the caecal region, allowing the products of digestion to pass through the gut while solid undigested

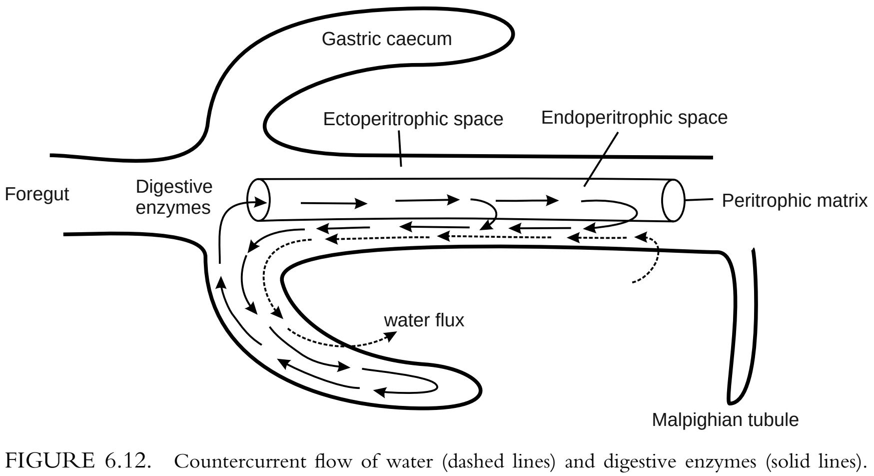


FIGURE 6.12. Countercurrent flow of water (dashed lines) and digestive enzymes (solid lines). Secretion of fluid by the posterior midgut is absorbed by the caeca. Enzymes that are involved in initial digestion pass through the peritrophic matrix, and those involved in the later stages of digestion remain in the ectoperitrophic space and on the surface of midgut cells. The countercurrent flow allows the products of digestion to pass through the gut and undigested food to move backward within the gut.

food moves backward. The caeca may be associated with mechanisms of food detoxification that allow insects to ingest plant materials that contain potentially toxic secondary compounds.

Because it lacks a cuticular lining, the cells that make up the midgut are susceptible to abrasion by food. Depending on the insect, either certain anterior midgut cells or all the cells of the midgut produce a **peritrophic matrix** (PM) that consists of a network of chitin microfibrils within a matrix of carbohydrate and protein. The various proteins that make up the PM are referred to as **peritrophins** and have chitin-binding domains. The chitin precursors and proteins are secreted by midgut cells, and the PM is self-organized by the interlocking chitin fibers and peritrophins. When secreted by all midgut cells, the PM condenses to form the matrix that surrounds the bolus of food. This **type I** PM is commonly produced as a delamination from the entire midgut epithelium, either continuously or in response to feeding. It is found in dictyopterans, orthopterans, coleopterans, many lepidopterans, adult female mosquitoes, and some hymenopterans. The **type II** PM, found in some dipterans and a few lepidopterans, is continuously formed by a ring of cells at the anterior region of the midgut, forming a tube that moves backward to enclose the gut contents. The **cardia**, or stomodeal valve, is a specialized fold of the proventriculus that serves as a press to squeeze the components to form the tube. The PM may be produced differently during the different life stages of the same insect. Mosquito larvae produce a type II PM, while the adults produce a type I PM. Besides their methods of formation, there do not appear to be significant differences between the two types at the structural or molecular levels. Many insects lack a PM, including some adult ants and most adult lepidopterans.

A PM is found in some fluid-feeding insects that do not ingest food particles that could abrade the gut cells, but here it may have a more important function in the creation of compartments for digestion. The PM contains pores and is permeable to some digestive enzymes and the products of digestion. This selective permeability creates a compartmentalization into an **endoperitrophic space** surrounded by the PM and an **ectoperitrophic** space that lies between the midgut wall and the PM (Figure 6.12). These compartments may also aid the caeca in maintaining the countercurrent water flows that result in the movement of solutes in the gut and a compartmentalization of digestive enzymes and products, with large enzymes restricted to the ectoperitrophic space. Fluid carrying the products of digestion moves forward through the ectoperitrophic space as a result of the absorption that occurs in the anterior midgut and gastric caeca. The PM can serve as a barrier to parasites such as the microbial control agent, *Bacillus thuringiensis*, and its impermeability can protect the insect against toxins in food to prevent them from crossing the PM and directly affecting the midgut cells. Moreover, it can protect herbivorous insects from oxidative damage that comes from ingested allelochemicals by binding the toxic material and scavenging

dietary oxidants. The ookinete stage of the malaria parasite *Plasmodium* produces a chitinase that aids in penetration of the PM and allows it to invade the midgut epithelium.

To accommodate the large amount of water in the diet, cicadas and cercopid Homoptera have a gut modification known as a **filter chamber** (Figure 8.15) in which the anterior midgut is expanded to wrap around the posterior midgut and the proximal ends of the Malpighian tubules, and the entire structure is enclosed in a cellular sheath. Water entering the anterior midgut can thus pass directly to the Malpighian tubules for excretion, so it is not absorbed and the hemolymph does not become diluted.

Hindgut

The insect hindgut, along with the Malpighian tubules, is primarily concerned with osmoregulation and is discussed in more detail in Chapter 8. The Malpighian tubules produce a primary iso-osmotic urine that is rich in potassium, low in sodium, and contains various ions, amino acids, and waste materials. The hindgut is capable of a selective resorption of the amino acids, water, and ions and produces a hyper- or hypo-osmotic urine that is deposited to the outside. Undigested food and waste products from digestion also pass through the hindgut, which can recover a number of important substances leaving a dehydrated fecal pellet for excretion. The hindgut may be differentiated into a **pylorus**, **ileum**, and **rectum**. The pylorus is a valve between the midgut and hindgut and the region where the Malpighian tubules arise. The ileum usually is a narrow tube but may be expanded into a fermentation chamber in insects that accommodate symbionts. The rectum is an enlarged region that may contain **rectal pads** that are columnar, in contrast to the usually flattened epithelium, and are involved in fluid transport. The anterior region of the rectum is enlarged into a brachial chamber in dragonfly larvae that is richly tracheated and serves as a gill (see Chapter 9). Although it is derived from an invagination of epidermal cells, the inwardly secreted cuticle of the hindgut is many times more permeable than the lining of the foregut.

Some Considerations for Insect Digestion

An important consideration in understanding the adaptive features of the insect digestive tract and its morphological and physiological variation is the particular insect's phylogenetic position and the diet of its ancestors, rather than what it currently happens to eat. The true generalists are typified by the cockroaches, which eat a wide variety of foods and have a gut that is divided evenly between storage, digestion, and osmoregulation. Two basic feeding strategies have shaped

the morphological evolution of the insect gut: whether the insect feeds on solid or liquid food, and whether it feeds on either animals or plants.

Ingested food mixed with salivary secretions undergoes preliminary digestion in the crop and moves backward into the midgut for further digestion and absorption. Protein digestion and final digestion of carbohydrates occurs in the midgut, which has a pH that is slightly acidic to neutral. The anterior region of the hindgut is adapted as a fermentation chamber and is alkaline in pH, conditions that favor the survival of populations of microorganisms. The rectum removes water from the feces and allows a dry fecal pellet to be deposited. The time it may take for solid food to pass through the entire alimentary canal varies tremendously, and within a species it may depend on nutritional history. Food is retained in the gut longer when the insect is starved. In the cockroach, solid food requires about 20 h to pass.

Plant feeding is considered to be a more advanced specialization. Insects that feed exclusively on plants generally ingest suboptimal levels of nutrients and are forced to process tremendous volumes to gain sufficient amounts of necessary components. The food, together with saliva, travels through the short foregut to the midgut for absorption. No digestion appears to occur in the foregut of Lepidoptera larvae, with initial digestion taking place within the endoperitrophic space of the midgut. The circulation of digesting food between the endo- and ectoperitrophic spaces is driven by fluid fluxes produced by the columnar cells in the anterior and posterior midgut and the gastric caeca. Liquid plant feeding insects, primarily those in the Homoptera and Hemiptera, ingest plant juices that contain dilute nutrients along with large amounts of water. The gut is generally much longer than the body to provide for the processing of the large amounts of dilute fluids. The gut pH is usually high, with the alkaline conditions protecting the insects from the toxic secondary substances that are ingested with the meal. The cibarium of these insects is often equipped with a muscular pump to suck up the liquids.

The general nutrition requirements of insects vary tremendously with species and physiological state, and the type of food ingested at different times of the life cycle can also vary enormously. Many insects choose diets that provide an optimal balance of the nutritional components that are required. If the nutrients from ingested food failed to match the proportions of nutrients required, the remaining components that were not utilized would have to be excreted at a cost that would weigh heavily against the insect's fitness. *Aedes aegypti* mosquitoes that feed on guinea pigs produce more eggs per volume of blood ingested than they produce when they feed on humans. The difference is because of the higher concentrations of the amino acid isoleucine in guinea pig blood. The remaining amino acids that are not utilized because of the missing isoleucine are not metabolized and are excreted. In designing insect diets, it is therefore important not only to include the essential components but to include them in the proper proportions.

The microbial flora that inhabits a typical insect gut probably outnumbers the total cells of the insect. The contributions of these microorganisms have not been thoroughly examined, perhaps because it is difficult to establish the resident organisms from those transient inhabitants that are simply passing through. True symbiotic relationships are considered when the microorganisms provide a novel metabolic pathway that otherwise may not occur and are always present in stable populations within certain parts of the gut and in certain stages of the insect. Gut microorganisms may provide a nutritional role by supplying digestive enzymes and vitamins, improving the efficiency of digestion, and allowing the insect host to better survive on a suboptimal diet. They can detoxify otherwise toxic plant components that are ingested, such as alkaloids and tannins. Obligate blood-feeders rely on their symbionts to provide them with factors necessary for growth and reproduction. Tsetse feed exclusively on an unbalanced diet of vertebrate blood and became associated with the obligate symbiont *Wigglesworthia glossinidia* about 80 million to 100 million years ago. The *Wigglesworthia* are located in specialized epithelial cells of the midgut and contribute to adult fecundity by providing B-complex vitamins that are otherwise not obtainable. A similar situation exists for the blood-sucking bug *Rhodnius prolixus* and its symbiont *Rhodococcus rhodnii*.

The ability of insect vectors to harbor human pathogens can be influenced by the presence of nonpathogens. Pathogenic microorganisms in the gut may be displaced by the normal resident flora. Flies and cockroaches harbor gut microorganisms that tend to be characteristic of their particular habitats, and interactions between established gut microorganisms can determine which may persist.

Digestion of Proteins

Proteins are broken down into their amino acid constituents by proteolytic enzymes and absorbed passively through the midgut wall when they are present in high concentrations. In lepidopteran larvae, low concentrations of amino acids are actively transported across the gut wall with different areas of the midgut responsible for taking up specific amino acids. The peptidases that initially digest the proteins may be **endopeptidases**, which cleave internal peptide bonds, or **exopeptidases**, which remove terminal amino acids from the protein chain. The most common endopeptidases are classified as serine proteases, including trypsin and chymotrypsin, which have serine at the active site. Trypsin cleaves protein chains on the carboxyl side of basic amino acids. Trypsin-like activity has been identified in most insect species. Chymotrypsin cleaves protein chains on the carboxyl side of aromatic amino acids. **Carboxypeptidases** are exopeptidases that remove the terminal amino acids from the carboxyl end of the chain. **Aminopeptidases** remove single amino acids from the N-terminal of

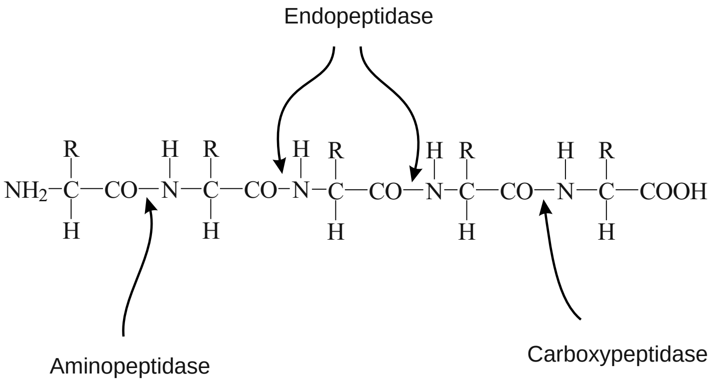


FIGURE 6.13. The action of aminopeptidases, endopeptidases, and carboxypeptidases on a polypeptide chain.

the peptide chain. They require metal ions for activity and generally have an alkaline pH optimum (Figure 6.13). Both types of enzymes may be present in the same insects.

In mosquitoes, the presence of initial digestion products in the blood induces the release of proteolytic enzymes by a **secretagogue** stimulus associated with the presence of the protein in the midgut. Two forms of trypsin are induced by blood ingestion: an early form in small amounts appears within 2h of the blood meal, followed by a late form in larger amounts that appears after 12h. The early form generates free amino acids that then induce larger amounts of the late form, which is responsible for most of the proteolytic activity in the gut (Figure 6.14). The transcription of the late trypsin gene depends on both the activity of the early trypsin as well as the quantity and quality of blood present.

Digestion of Carbohydrates

Glucosidases secreted in the midgut hydrolyze the glucosidic bonds between the sugar residues, and their specificity depends on the type of bond and whether the linkage is α or β. An α-glucosidase hydrolyzes the common α-glucosides of sucrose, maltose, trehalose, and melezitose; β-glucosidase breaks down the β-glucosides of cellobiose and gentiobiose; α-galactosidase acts on the α-galactosides melibiose and raffinose; β-fructosidase hydrolyzes the β-fructosides sucrose, gentianose, and raffinose. **Amylases** act on the α-glucosidic linkages in starch and glycogen (Figure 6.15). One of the most common carbohydrases in insects is **trehalase**, which hydrolyzes trehalose into two glucose molecules.

Although cellulose is common in the diet of phytophagous insects, the innate ability to digest it is rare. Three classes of enzymes are involved in the hydrolysis

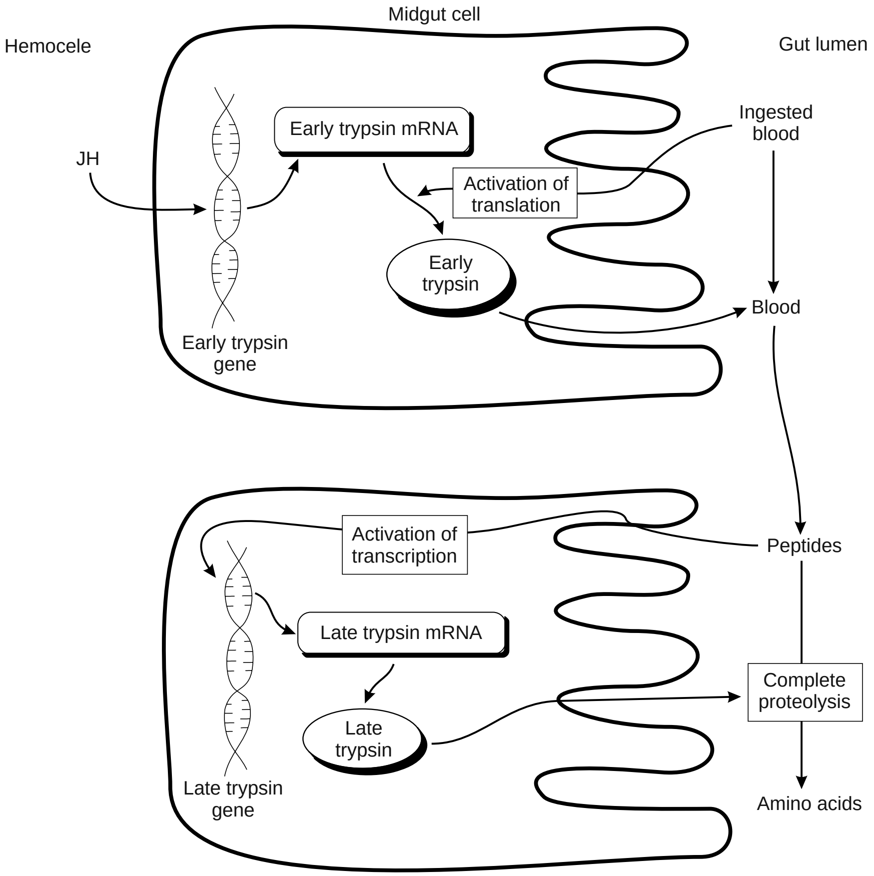


FIGURE 6.14. Mechanism of proteolytic digestion in mosquitoes. JH activates an early trypsin gene resulting in early trypsin mRNA. A newly ingested blood meal activates its translation to early trypsin, which digests the blood to peptides. The peptides activate transcription of late trypsin mRNA, and this late trypsin breaks the peptides down to amino acids.

of cellulose: endo-β-1,4-glucanases randomly cleave the β-1,4-glucosidic bonds in the cellulose chain; exo-β-1,4-glucanases cleave cellobioise residues from one end of the chain; and β-1,4-glucosidases break down cellobiose to glucose. These enzymes are usually produced by endosymbiotic microorganisms that live in the gut and not by the insects themselves. In the lower termites and wood roaches, a population of protozoa is established in the hindgut that digests cellulose. Bacterial symbionts are responsible for cellulose digestion in many higher termites, cockroaches, and beetles. Some fungus-growing termites and ants cultivate the fungi in gardens and ingest the fungal enzymes that are responsible for their ability to digest cellulose. Cellulose digestion that is independent of symbionts

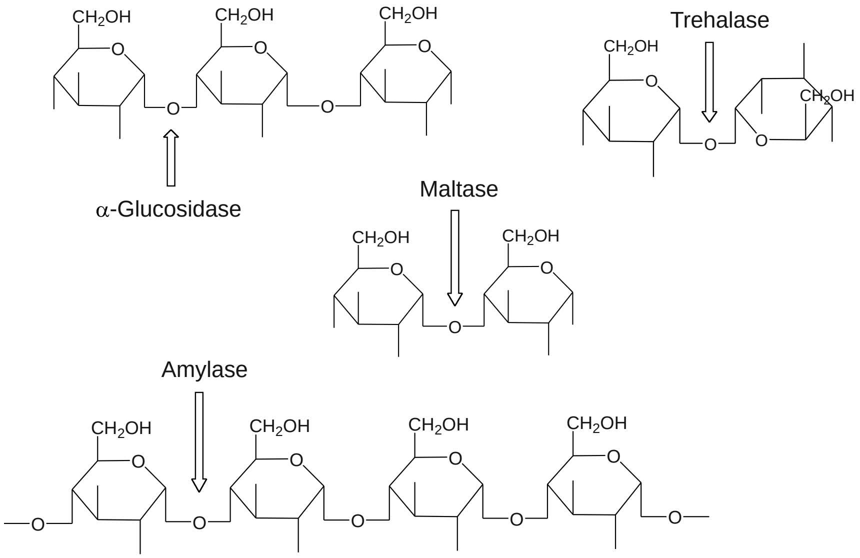


FIGURE 6.15. Enzymes that hydrolyze polysaccharides.

has only been identified in cerambycid beetle larvae, in silverfish and firebrats, and in a few species of higher termites and the Australian wood-eating cockroach, *Panesthia cribrata*. The metabolism of cellulose by termites is an important source of atmospheric methane.

Carbohydrates are ingested in the food as polysaccharides and disaccharides and are broken down to monosaccharides for absorption through the gut wall. The absorption of monosaccharides generally occurs passively through the midgut wall, but there is some evidence that this diffusion is facilitated by an active glucose transporter. The sugars that pass into the hemolymph are then taken up by the fat body cells that surround the gut. Diffusion requires a concentration gradient to direct the movement of sugars from the midgut into the hemolymph. To maintain this gradient, absorbed glucose is converted to the disaccharide trehalose in the hemolymph to accommodate the passage of more glucose from the midgut.

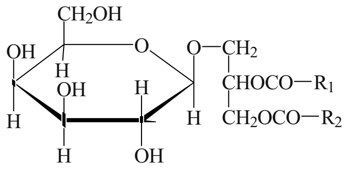
Digestion of Lipids

Insects may ingest storage lipids, found in seeds and fatty tissue of animals, and membrane lipids that are found in all cells. Triacylglycerols are the primary storage lipid in seeds and adipose tissue of animals. Phytophagous insects ingest

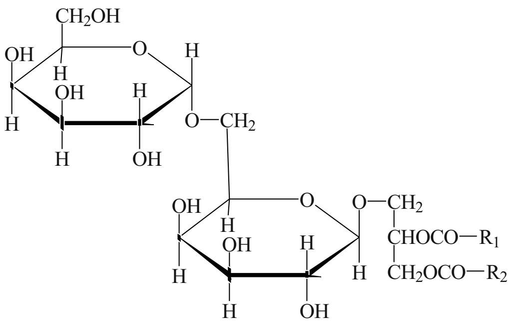
mostly the plant constituents monogalactosyl diglycerides, found mainly in chloroplasts, and digalactosyl diglycerides (Figure 6.16). The major types of lipids in animals are triacylglycerols, phospholipids, and cholesterol (Figure 6.17). Membrane lipids include the phospholipids and glycolipids. The lipids are absorbed both as fatty acids or diacylglycerols in the anterior midgut and gastric caeca. In the cockroach, *Periplaneta*, some absorption of lipids also occurs in the cuticle-lined crop.

Although lipids are insoluble in water, they must diffuse through the aqueous hemolymph in order to be absorbed. In vertebrates, bile produced in the liver solubilizes the lipids in the gut, but no such emulsifiers have been identified in insects. Instead, dietary lipids may first be incorporated into polar fractions that increase their solubilization for absorption.

Lipolytic digestive enzymes have not been well-studied in insects, and the process by which triacylglycerols in the diet are broken down and transferred to the hemolymph through the gut is largely unknown. In general, **phospholipases** remove the fatty acid portion from phosphatides. They break down the cell membranes of ingested food, allowing other enzymes to act on the cell contents. **Triacylglycerol lipases** hydrolyze the outer ester links of triacylglycerols only at the interface of water and lipid. **Esterases** act on molecules that are completely dissolved in water, hydrolyzing carboxyl esters into alcohol and



Monogalactosyl diglyceride



Digalactosyl diglyceride

FIGURE 6.16. The major diglycerides found in plants.

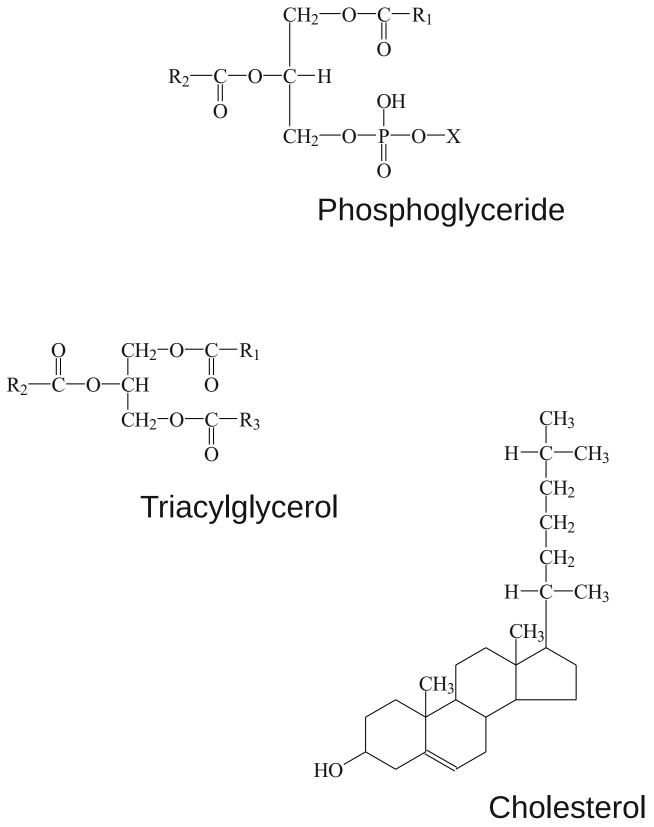


FIGURE 6.17. The major types of animal lipids. X = alcohol in phosphoglyceride.

carboxylate. They may break down cholesterol and are important in the resistance to insecticides and plant secondary substances.

The major lipid components that appear in the hemolymph are diacylglycerols. These are resynthesized by midgut cells from digested components before they are released into the hemolymph. Insoluble in the aqueous hemolymph by themselves, the diacylglycerols are bound to a **lipophorin** that allows them to be transported throughout the body. Lipophorins also transport the cholesterol and phospholipids that are present in the hemolymph. This lipid transport will be described further in the section on the metabolism of lipids.

METABOLIC PROCESSES IN INSECTS

Metabolism is differentiated into the two processes of catabolism and anabolism. **Catabolism** involves the enzymatic degradation of large nutrient molecules from

an organism's reserves or from the environment. **Anabolism** is the enzymatic synthesis of larger cell components from smaller precursors. Whenever molecules are degraded or synthesized, there is a change in the energy states between the starting substrates and ending products, and living systems can capture this change in energy or store it in molecules that conserve the energy as phosphate bonds. By coupling these degradative, energy-releasing steps with equivalent energy-conserving steps, the difference in energy can be transferred efficiently without generating too much heat that is generally wasted energy.

Adenosine triphosphate, or **ATP**, is the universal energy currency of cells, serving as a transfer medium for energy and its temporary storage. The energy derived from the stepwise oxidation of food is harnessed and parceled out by ATP to perform cellular work. The ATP molecule consists of adenine, ribose, and a triphosphate unit (Figure 6.18). The phosphate bonds are what give ATP its ability to store energy, which is liberated when it is hydrolyzed to adenosine diphosphate (ADP). The turnover of cellular ATP is very high, as it is typically utilized shortly after it is formed. There are other high-energy nucleotides that play an equally important but less major role in the transfer of cellular energy, including **guanosine triphosphate** (**GTP**) and **uridine triphosphate** (**UTP**).

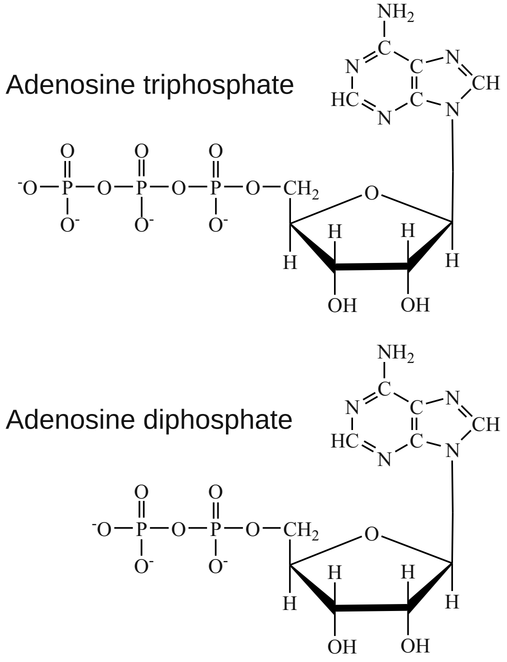


FIGURE 6.18. The molecules used most for energy transfer within the cell.

The pathways by which ingested and stored carbohydrate, fat, and protein are catabolized involve the transfer of energy in small amounts during their degradation or synthesis. The energy locked up in organic molecules is released primarily by **oxidation**, the removal of electrons from the molecules and the transfer of those electrons to other molecules within the cell that undergo **reduction** when they acquire the electrons. This generation of energy occurs in three general stages in all animals. In the first stage, the large food molecules are converted into smaller ones within the alimentary tract. We have already seen that proteins are degraded to amino acids, fats to fatty acids and glycerol, and polysaccharides to monosaccharides (Figure 6.19). No energy is produced during this stage, but in fact, some may be consumed in the process of synthesizing enzymes needed to break the large molecules down. In the second stage, these simpler molecules are further broken down to two carbon molecules that are acceptable for entry into the citric acid cycle. These molecules consist primarily of acetic acid that is combined with coenzyme A to form acetyl coenzyme A. A small amount of energy is generated anaerobically in this stage with the production

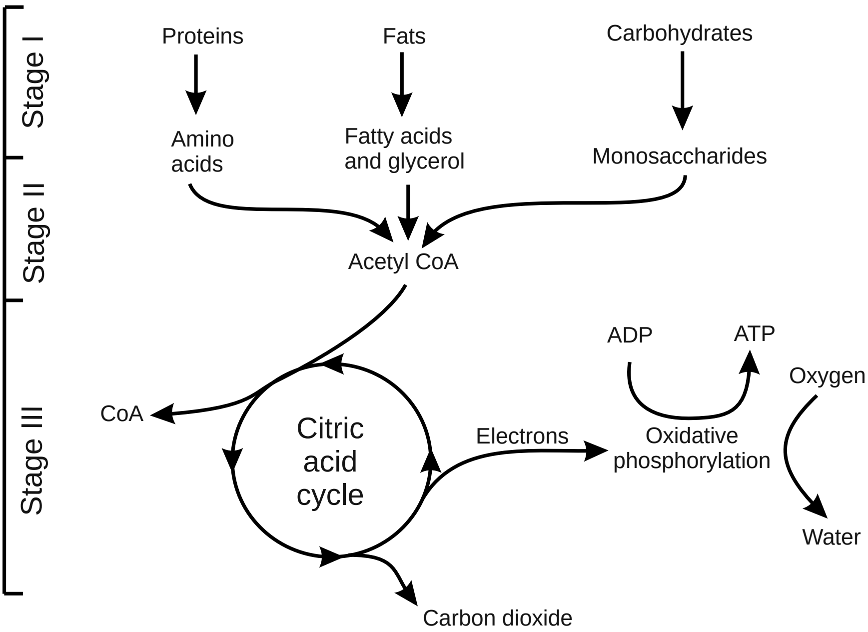


FIGURE 6.19. Stages in the oxidation of food. In stage I, proteins, fats, and carbohydrates are broken down into their constituents. In stage II, these building blocks are reduced to two carbon molecules for entry in the citric acid cycle. In stage III, the two carbon molecules enter the citric acid cycle, with carbon dioxide and water produced along with the bulk of the energy transfer to ATP.

of a few molecules of ATP and carbon dioxide. In the third stage, the molecules enter the citric acid cycle where they are oxidized to carbon dioxide and their electrons are ultimately transferred to oxygen to form water. It is during this process of oxidative phosphorylation that much of the energy is coupled to the generation of large amounts of ATP.

The difference in energy resulting from the electrons being removed from food is eventually transferred to ATP by the oxidation reactions. Electrons are shuttled within the cell and are ultimately passed to oxygen, resulting in the formation of water and the synthesis of ATP. The major electron acceptor molecules during food oxidation are **nicotinamide adenine dinucleotide** (NAD^+) and **flavin adenine dinucleotide** (FAD) (Figure 6.20). Successive decarboxylations of the food substrate also account for the carbon dioxide that is released into the environment as cells respire. During the degradation of proteins, nitrogen is additionally removed from the molecules and released as ammonia. Because ammonia is so toxic, most terrestrial animals incorporate the nitrogen into more complex but less toxic molecules such as urea and uric acid. The net result of these biochemical transformations is the production of useful energy from the degradation of organic molecules. Energy that is not required immediately is stored as trehalose, glycogen, or fat.

Metabolism of Carbohydrates

The most important carbohydrate reserves in insects are glycogen and trehalose, synthesized when the intake of carbohydrate is greater than what is immediately required. Both can easily be converted to glucose when the reserves need to be mobilized (Figure 6.21). Glycogen is a polymer of many glucose residues existing as a branched chain storage form (Figure 6.22). Supplies of glycogen are stored in the flight muscles, fat body, and around the digestive tract. Because it is able to be stored within cells, glycogen can provide an immediate source of energy for rapidly respiring muscles, such as the flight muscles. Glycogen stored in the fat body can be immediately converted to trehalose for release into the hemolymph.

The mobilization of glycogen reserves to form glucose and trehalose in the hemolymph is hormonally controlled by **hypertrehalosemic hormone** (HrTH) released from the corpus cardiacum. Hormone release is triggered by the declining concentrations of hemolymph sugars as they are consumed during metabolism or during starvation. Through a second messenger system, HrTH activates the normally inactive enzyme, phosphorylase kinase, that then activates **glycogen phosphorylase** to cause glycogen-1-phosphate to be released from glycogen and the ultimate formation of trehalose. Because in many insects, **adipokinetic hormone** (AKH) additionally mobilizes glycogen reserves as well as lipid and is structurally related to HrTH, HrTH is considered to be a member

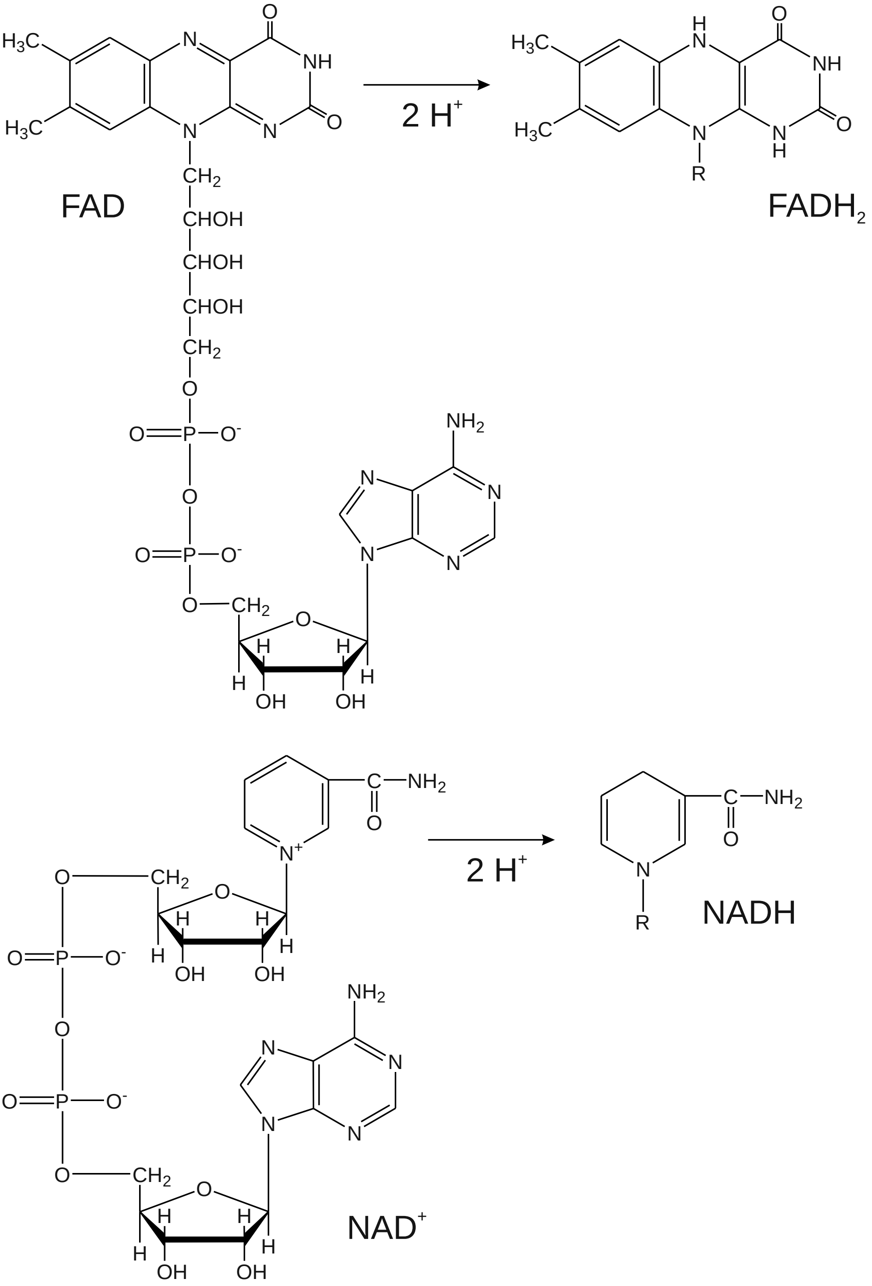


FIGURE 6.20. Oxidized and reduced forms of the major electron acceptor molecules.

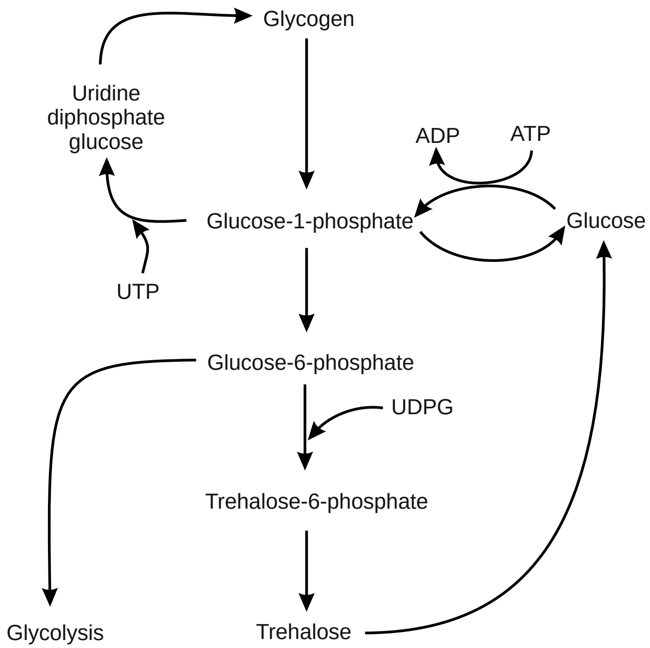


FIGURE 6.21. Interconversions of glycogen, glucose, and trehalose. Glycogen is the storage form of carbohydrate and is converted to glucose and trehalose when energy is required.

of the larger adipokinetic hormone family that contains close to 40 isoforms. AKH and its activity is discussed in more detail in the section on lipid metabolism. The biogenic amine **octopamine**, acting as a neurohormone, also activates glycogen phosphorylase. Insects have evolved numerous pathways for the mobilization of energy substrates.

Trehalose, a nonreducing disaccharide of glucose, is the major hemolymph sugar in insects, synthesized by the fat body by combining the two intermediates of glycolysis: glucose-1-phosphate and glucose-6-phosphate. It serves as a circulating energy source, as glucose does in the blood of vertebrates (Figure 6.22), but trehalose concentrations in insect hemolymph are considerably higher, ranging from 0.5 to 5.0 g/100 ml, compared to the levels of less than 0.1 g/100 ml of glucose in vertebrate blood. As a disaccharide, it is a larger molecule than glucose and diffuses more slowly, so trehalose can be maintained at a higher concentration in the hemolymph before it diffuses across membranes and into cells. Trehalose also accounts for reduced osmotic effects compared to the same concentrations of the monosaccharide glucose. The higher concentration of hemolymph trehalose that can be maintained facilitates its distribution by diffusion to all the cells of the insect.

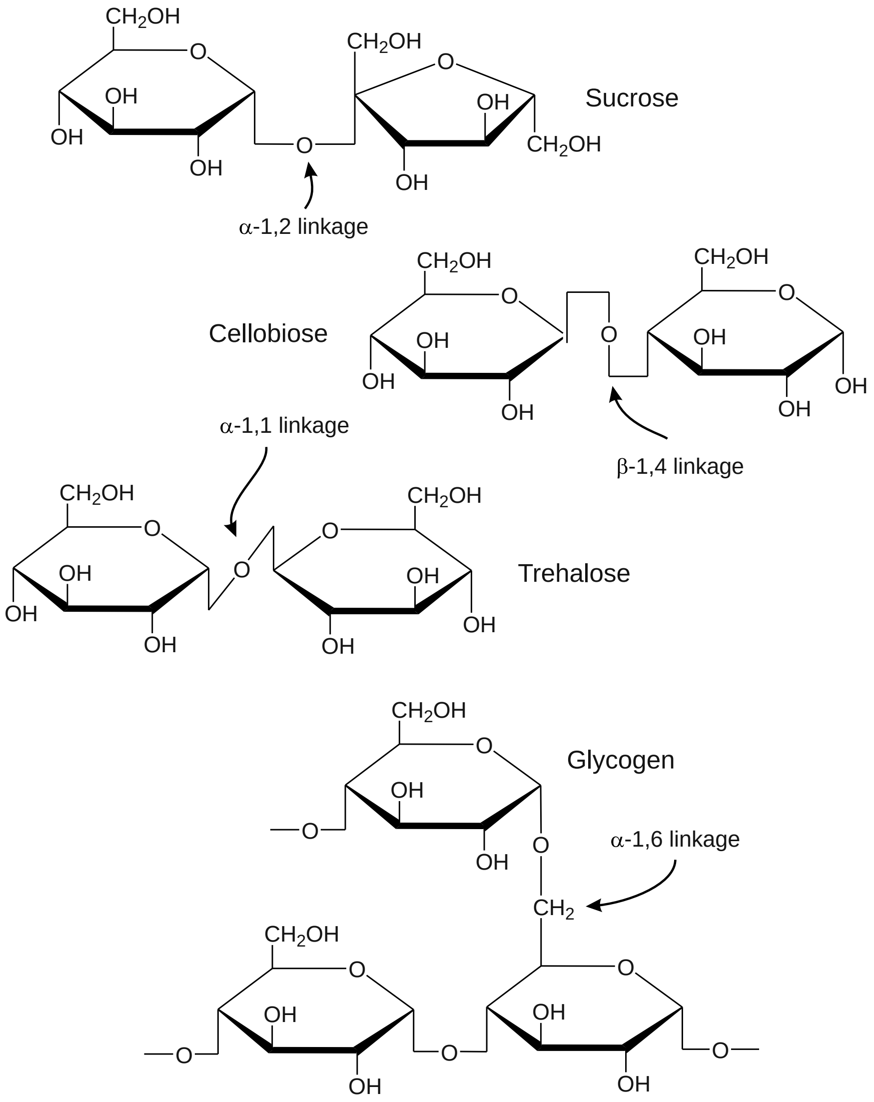


FIGURE 6.22. Some of the major carbohydrates found in insects.

Another reason for the utilization of trehalose rather than glucose in insects relates to the ease with which glucose in the diet is absorbed through the gut wall. If high levels of glucose were maintained in the hemolymph, they would interfere with the uptake of glucose from the gut because the uptake occurs primarily by passive diffusion. By maintaining a lower concentration of glucose in the hemolymph, the uptake of glucose from the gut is facilitated. Once

in the hemolymph, the glucose is then converted to trehalose by the fat body. Other cells can readily hydrolyze the trehalose to glucose that is oxidized to provide energy. Trehalose also functions as a cryoprotectant, decreasing the supercooling point of the insect and stabilizing cell membranes and protecting them from rupture. Other sugars such as glucose and fructose may also be found in the hemolymph at lower concentrations. Generally, the presence of sugars in the hemolymph is unregulated and simply reflects the metabolic rate and physiological state of the insect.

The first step in the generation of metabolic energy from carbohydrate is **glycolysis**, the sequence of reactions that breaks glucose down to pyruvate with the accompanying generation of ATP. The process of glycolysis occurs in nearly all cells and serves as a prelude to the more complete breakdown of the molecules into carbon dioxide and water in the citric acid cycle. The basic steps of glycolysis and the enzymes involved are no different in insects than in other organisms. The process occurs in the cell cytoplasm, generating two molecules of ATP and two molecules of pyruvate for every molecule of glucose. The subsequent fate of pyruvate can vary depending on the organism and the circumstances. In yeast and a few other microorganisms, pyruvate is broken down into ethanol and CO_2 (Figure 6.23). During intense metabolic activity when oxygen is limited, as in the skeletal muscles of insects and vertebrates during exertion, pyruvate is converted to lactic acid. This regenerates NAD^+ and buys the organism some time that allows glycolysis to continue. Pyruvate can also be transaminated to α-ketoglutarate by glutamate in those insects that use proline as fuel for flight. This pathway is described in more detail under the metabolism of proteins. Because each molecule of glucose gives rise to two molecules of pyruvate, a total of four molecules of ATP results from glycolysis, but after subtracting the two molecules of ATP that are used, a net production of two molecules of ATP and two molecules of NADH results.

More energy can be extracted from glucose when oxygen is plentiful if the pyruvate is converted to acetyl CoA that is able to enter the oxidative pathway of the citric acid cycle. When sufficient oxygen is present, the pyruvate that is first converted to acetyl CoA enters the mitochondria to participate in a series of oxidative reactions called the **citric acid cycle**. In addition to the oxidation of pyruvate, the citric acid cycle provides a mechanism for the interconversion of many biochemical intermediates. Here the acetyl CoA is oxidized to carbon dioxide and water, and the electrons are passed through an electron transport chain with their final acceptor of oxygen. The energy that is released during this transfer is used to phosphorylate ADP to ATP (Figure 6.24).

A unique pathway of carbohydrate metabolism is found in some insects that rely on carbohydrate substrates to produce energy for flight. Glycolysis takes place in the cytoplasm, and electron transport occurs in the mitochondria, but because of the impermeability of the mitochondrial membrane, pyridine nucleotides such as NAD^+ are prevented from passing between the mitochondrion and

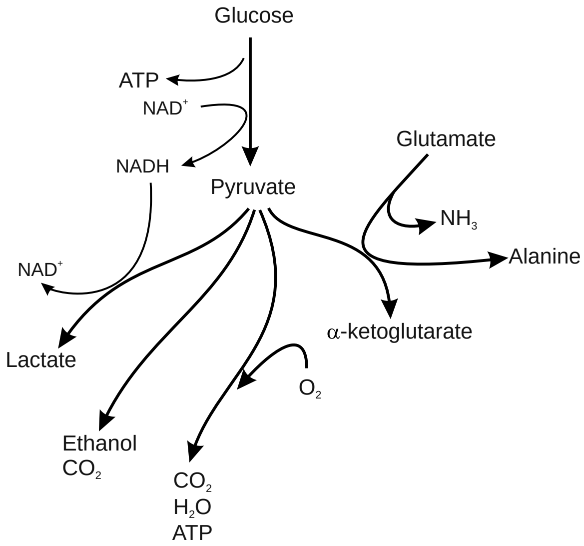


FIGURE 6.23. The fate of pyruvate in respiring tissues. In yeast, pyruvate is broken down into carbon dioxide and ethanol. In insect and vertebrate skeletal muscles when oxygen is limited, the pyruvate is broken down to lactate. In the presence of sufficient oxygen, the pyruvate is converted to acetyl CoA and enters the citric acid cycle where it is released as carbon dioxide and water, and large amounts of ATP are produced. In some insects, the pyruvate may be transaminated by glutamate to α-ketoglutarate in a pathway that utilizes proline for flight energy.

the cytoplasm. Thus, the NADH that is formed with the oxidation of glyceraldehyde-3-phosphate during glycolysis within the cytoplasm cannot become oxidized by the electron transport chain because it is unable to enter the mitochondrion, and without the regeneration of NAD^+, glycolysis cannot continue. The operation of the **glycerol-3-phosphate shuttle** in insects allows the reducing equivalents from the cytoplasmic pool of NADH to cross the permeability barrier of the mitochondrial membrane and be oxidized by the cytochromes, ultimately transferring their electrons to oxygen (Figures 6.25). The shuttle thus reoxidizes the NADH that is produced during the glycolysis occurring in flight muscles by carrying the electrons from NADH across the mitochondrial membrane rather than the NADH itself. One carrier is glycerol-3-phosphate, formed when NADH transfers its electrons to dihydroxyacetone phosphate. The glycerol-3-phosphate is able to cross the outer mitochondrial membrane where it is reoxidized to dihydroxyacetone phosphate by the electron acceptor FAD that transfers its electrons to the respiratory chain. The dihydroxyacetone phosphate diffuses back into the cytoplasm to complete the shuttle. Other insect skeletal muscles lack this pathway, and there the NAD^+ is regenerated by the reduction of pyruvate to lactic acid. The lactic acid is a metabolic

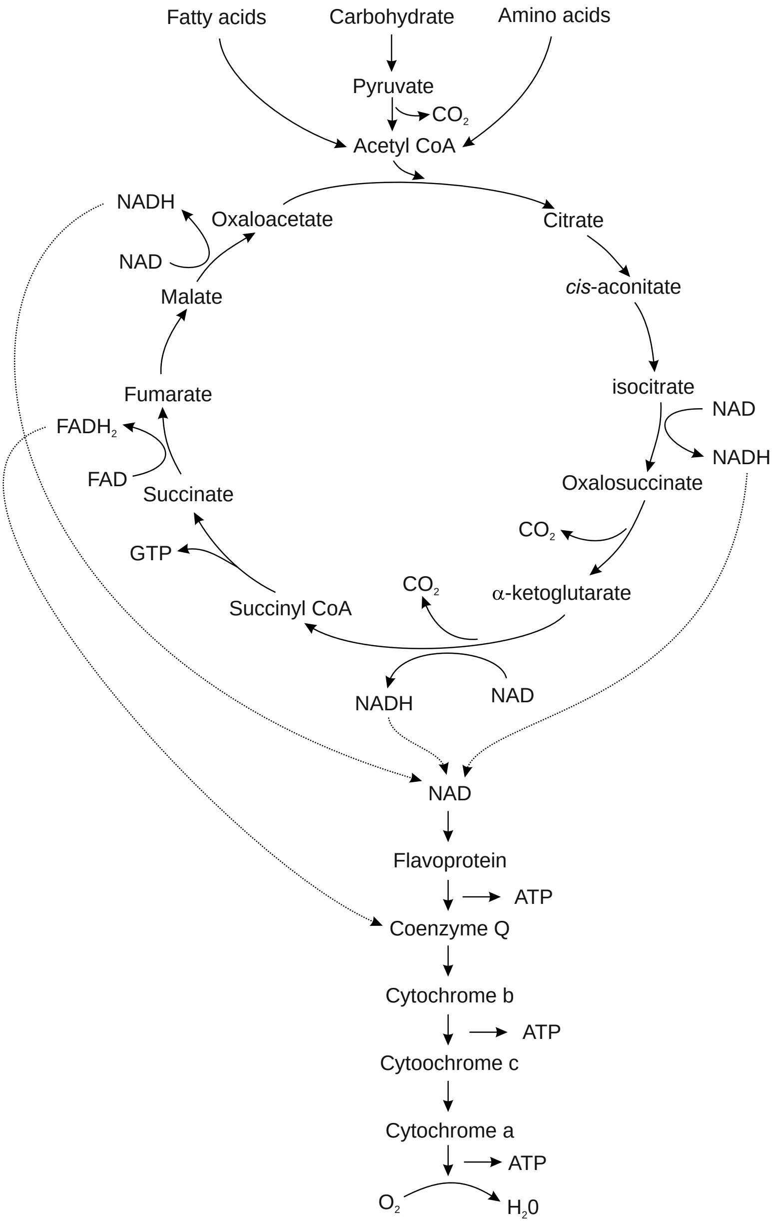


FIGURE 6.24. The intermediates of the citric acid cycle and the passage of electrons through the electron transport chain, where ATP is generated.

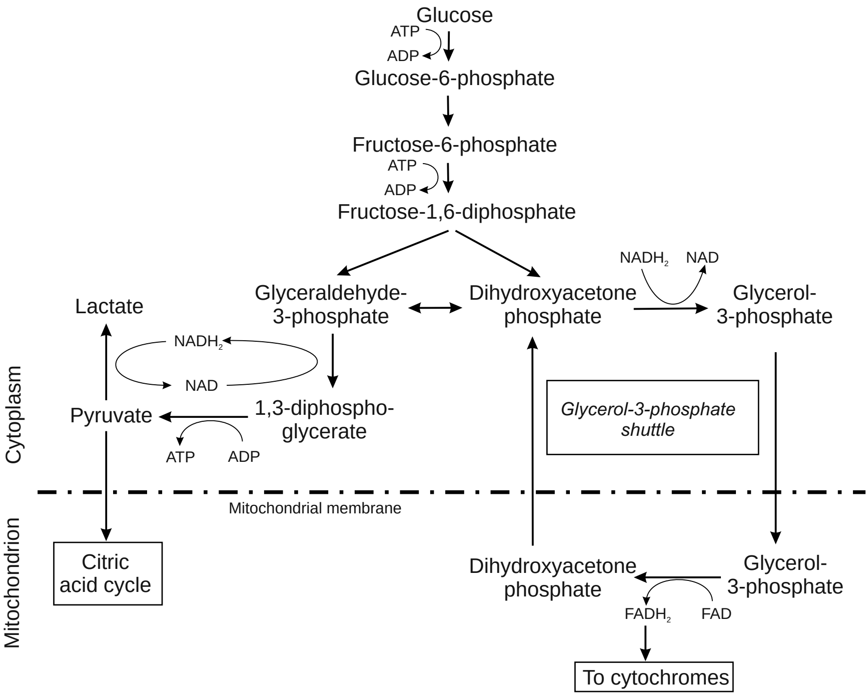


FIGURE 6.25. The glycerol-3-phosphate shuttle that operates in insect flight muscles.

dead end; it must be converted back to pyruvate before it can be metabolized, but the reduction of pyruvate at least generates the NAD^+ that allows glycolysis to continue. The glycerol-3-phosphate shuttle prevents the wasteful formation of lactic acid in rapidly respiring insect tissues when oxygen supplies might be limited.

Chitin is a major carbohydrate component of the insect procuticle, often comprising half the dry weight of the cuticle. Most cells that have an epidermal origin produce chitin. It is a polymer of *N*-acetyl-D-glucosamine residues (some glucosamine residues may also be present; see Figure 2.16) joined in one to four β linkages. The synthesis of chitin begins with glucose and involves a phosphorylation, amination, acetylation, and conjugation with uridine diphosphate (Figure 6.26). The mature helical polymer of chitin may consist of as many as 1500 *N*-acetyl-D-glucosamine residues, along with the occasional glucosamine. It can be degraded during the molting process by molting fluid and its raw materials recovered for use in the new cuticle that is synthesized. During the molt, the enzyme **chitinase** breaks the polymer down to chitobiose and *N*-acetyl-D-glucosamine.

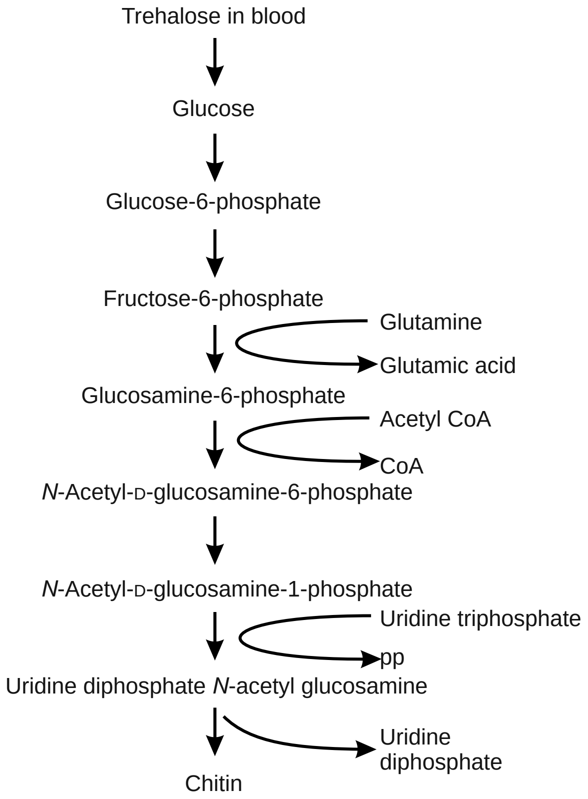


FIGURE 6.26. Steps in the synthesis of chitin from trehalose in the blood.

Metabolism of Proteins

The most important functions of amino acids, the building blocks of the proteins that are derived from the insect diet, include the synthesis of structural proteins of the integument and the synthesis of hormones and enzymes that participate in metabolic reactions. Some amino acids also participate in the synthesis of nucleic acids. The same 20 amino acids are involved in building the proteins of all living things from bacteria to vertebrates, and insects also employ these 20 amino acids in constructing proteins. Of these 20 amino acids, nine of them cannot be synthesized by the interconversion of other amino acids. These nine **essential amino acids** must be ingested as dietary components, whereas the remaining 11 others are **nonessential** and may be derived by biochemical conversions (Table 6.1, Figure 6.27). Insects need the same nine essential amino acids that vertebrates require. Because the central carbon atom allows a tetrahedral array of different groups that bond to it, two mirror image isomers that are

TABLE 6.1. Basic amino acids.

Essential	Non-essential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartate
Methionine	Cysteine
Phenylalanine	Glutamate
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Serine
	Tyrosine

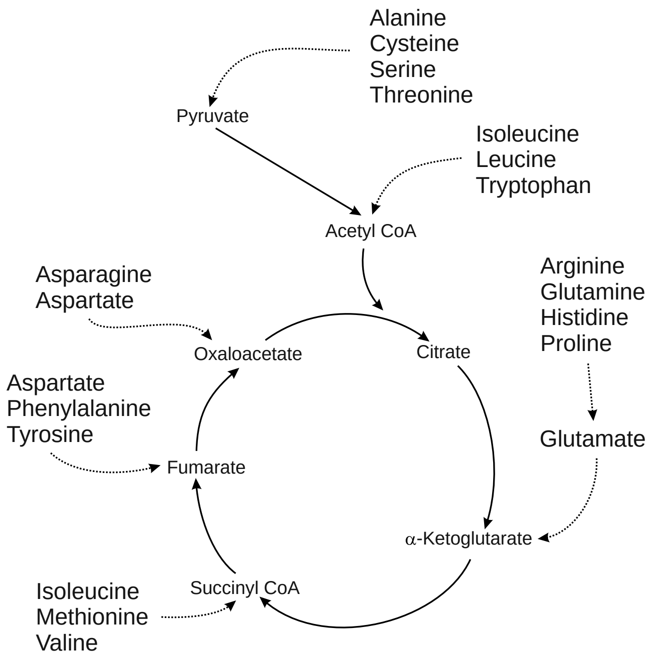


FIGURE 6.27. Interconversion of some amino acids and their entry points in the TCA.

designated D and L are possible. However, only the L isomers of amino acids are found in proteins.

Amino acids that are present in the hemolymph make a large osmotic contribution, sometimes accounting for more than 30% of its total osmotic activity. Amino acids also figure prominently in many biochemical pathways. Some

insects use the amino acid proline as a metabolic substrate for flight energy in addition to using fats and carbohydrates (see the next section on proline metabolism). Tyrosine is necessary for cuticular sclerotization, and glutamate is involved in neurotransmission. In insects, among the most important requirements for protein synthesis are for the proteins that will be deposited in the newly formed cuticle and that participate in cuticular sclerotization. The cuticular proteins are largely synthesized by epidermal cells, and the mechanisms involved are further discussed in Chapter 2. Another important protein consists of the vitellogenins that supply the protein requirements for the egg. The control of vitellogenin synthesis by cells of the fat body and ovarian follicle is discussed in Chapter 4. The production of both cuticular and vitellogenic proteins occurs in response to levels of juvenile hormone and 20-hydroxyecdysone.

In contrast to fats and carbohydrates that can easily be stored, the amino acids that are ingested in excess of the immediate needs of most animals are generally either used as metabolic fuel or excreted. However, many holometabolous insects have a particular need for proteins to be stored. The protein that is acquired during the larval stage must often be carried over to the pupal and adult stages that require protein but may be unable to acquire it. This protein is essential for metamorphosis, reproduction, and general body maintenance. Indeed, the consumption of the cast skin by some insects after molting is a way to recover some of the nitrogen that is lost during the molt.

There is a special family of proteins, the **storage hexamerins**, that has been described in many other arthropods besides insects. These proteins are members of a family of proteins that are each composed of six similar subunits of between 70 and 90 kDa. The hexamerins evolved from the copper-containing **hemocyanins** that are used to carry oxygen in the blood of crustaceans and chelicerate arthropods and originated more than 550 million years ago from oxygen-consuming phenoloxidases. However, as insects became terrestrial and evolved a tracheal system to bring oxygen to tissues, hemocyanins were no longer needed for respiration, and they lost their respiratory function. Immatures and adults of the more primitive stone fly, *Perla marginata*, do still retain a hexameric hemocyanin consisting of two subunit types of 659 and 655 amino acids, but in more advanced insects, hexamerins have lost the copper and no longer bind oxygen. Instead, they act primarily as storage proteins that provide amino acids that are required for protein synthesis in the developmental phases that do not feed. During the nonfeeding pupal stage, holometabolous insects depend on nutrients accumulated during the larval stages. The hexamerins are synthesized by the fat body and are released into the hemolymph during the larval stage; but just before metamorphosis, they are recaptured by the fat body and stored in cytoplasmic granules that can be utilized during adult development. Hexamerins may also be employed during diapause. The uptake of hexamerins by the fat body is induced when the rise in ecdysone titer at metamorphosis activates the receptor gene. Hexamerins may also serve as JH binding proteins, and in termites, hexamerin

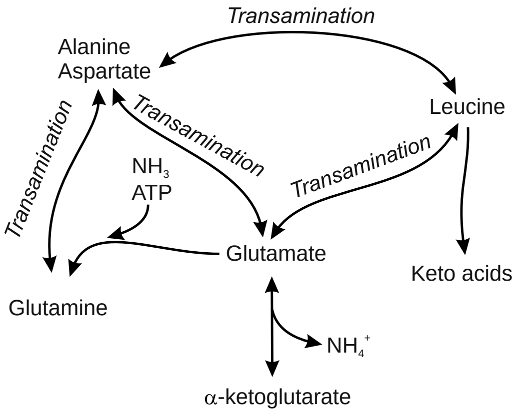


FIGURE 6.28. Transamination, the transfer of amino groups from amino acids to keto acids.

genes are induced by the rising titers of JH during caste differentiation and appear to regulate the development of castes.

The **lipophorins** are lipoproteins found in the hemolymph that serve as a vehicle for the transport of lipids that are otherwise not soluble in the aqueous blood. They have been found in all life stages of all the insect species that have been examined. They are loaded with the dietary lipid that is absorbed through the midgut wall and carry it to developing tissues or to the fat body where it may be stored. Juvenile hormones may also be transported by lipophorins. These are discussed in more detail in the section on lipid metabolism presented later in this chapter.

The degradation of dietary amino acids generally yields acetyl CoA, pyruvate, or other citric acid cycle intermediates. A common reaction in amino acid metabolism is **transamination**, in which the amino groups are transferred from amino acids to ketoacids for conversion into ammonia (Figure 6.28). The amino acids glutamate, aspartate, and alanine and their corresponding ketoacids are mostly involved, with glutamate serving as one of the key intermediates. Keto-acids can also be formed by an oxidative deamination. Amino acid degradation can thus commonly form metabolic intermediates that can be converted to glucose or enter the citric acid cycle.

Proline Metabolism

Many insects use carbohydrates and lipids to fuel flight, but some insects use the amino acid proline. Proline utilization may have been favored in some insects because its high solubility allows it to accumulate to high levels in the hemolymph to make it readily available to all tissues, and it does not require any

carrier molecules as do the lipids. Many insects that utilize proline lack extensive carbohydrate reserves, whereas others may use proline and carbohydrate equally. The tsetse, Colorado potato beetle, dung beetle, and *Aedes aegypti* mosquito synthesize proline in the fat body that is transported to and utilized by their flight muscles, which operate aerobically as a result of the efficient tracheal system. The preflight warmup of the fruit beetle, *Pachylomerus femoralis*, utilizes proline to provide energy.

The mechanism uses proline to transport acetyl units from acetyl CoA in the fat body through the hemolymph to the muscles. The α-ketoglutarate from the citric acid cycle in fat body cells is converted to proline, which in flight muscles is converted back to α-ketoglutarate that enters the citric acid cycle there. The proline is converted to glutamate by the enzyme proline dehydrogenase, which is activated by high levels of pyruvate, and the transamination of the glutamate with pyruvate produces the alanine and additionally α-ketoglutarate. The alanine is taken up by fat body cells and reconverted to proline, whereas the α-ketoglutarate enters the citric acid cycle. The α-ketoglutarate is further metabolized to oxaloacetate, and its condensation with acetyl CoA yields citrate. This allows the complete oxidation of pyruvate through the citric acid cycle, supplying citric acid cycle intermediates to prime the cycle and speed it up (Figure 6.29). The conversion of one mole of proline into one mole of alanine by this

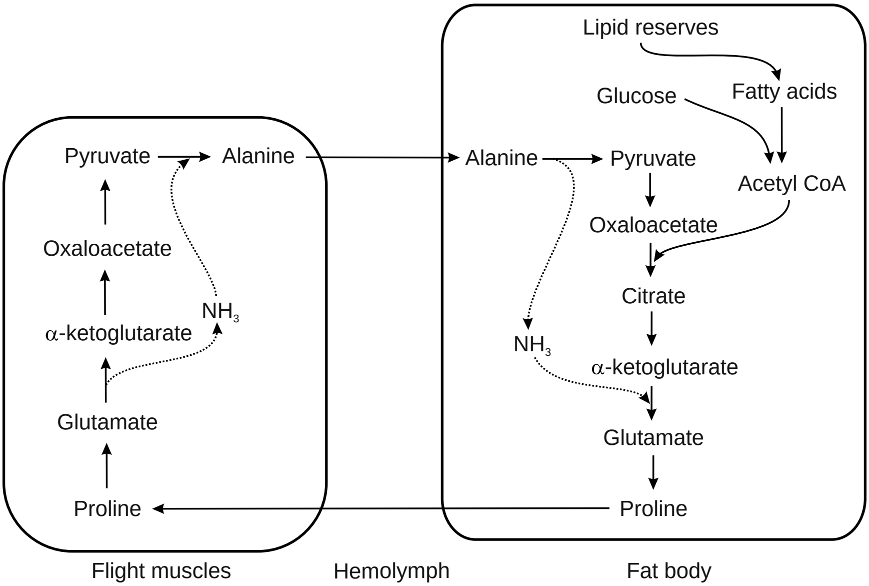


FIGURE 6.29. Utilization of fat body proline for flight.

mechanism yields 14 moles of ATP, comparing favorably to the 15 moles of ATP produced by one mole of pyruvate passing through the citric acid cycle. The high solubility of proline allows it to accumulate to high concentrations in the hemolymph and flight muscles to provide a readily available substrate for flight.

Vitamins

Vitamins are organic molecules that are required in the diets of animals in minute amounts, often serving as cofactors for metabolic enzymes. They can be divided into one of two groups based on their solubility in water. The water-soluble vitamins include those in the vitamin B complex and vitamin C. The fat-soluble vitamins are designated by the letters A, D, E, and K. It has been difficult to determine the various vitamin requirements of insects because trace quantities can be carried over from the larval stages or supplied by symbiotic microorganisms, and few generalities can be made. Vitamin A, or β-carotene, is an essential component of insect visual pigments and is required as a precursor for retinol. Other carotenoids contribute to body color as cuticular pigments and serve as general antioxidants. In addition to acquiring these from their diets, females may receive them from males in seminal fluid or the spermatophore. Vitamin E, or α-tocopherol, enhances life span and fecundity in some insects. L-carnitine is an essential water-soluble "quasi-vitamin" formerly called vitamin B_T because its activity was first identified in the beetle, *Tenebrio*. Carnitine transports long-chain fatty acids across the mitochondrial membranes so they can be processed by β-oxidation to yield ATP.

Metabolism of Lipids

Lipids are a heterogeneous group of compounds that are defined by their insolubility in water and high solubility in nonpolar organic solvents. Fatty acids are lipids that contain a long hydrocarbon chain and a terminal carboxylate group. They vary in chain length and the degree of unsaturation, or double bonds, within the chain, but those in biological systems usually contain an even number of carbon atoms and are unbranched. The physical characteristics of fatty acids and the lipids that are derived from them are largely based on the degree of saturation and the length of their chains. Those with shorter chains and more unsaturation tend to be more fluid in biological systems.

Fatty acids are significant molecules in biological systems. They are building blocks for the synthesis of the phospholipids that are important constituents of the cell membrane. They also act as hormones and as sources of metabolic energy that can be mobilized to meet the energy requirements of the insect. They make up a large part of the cuticular lipids that protect insects from desiccating. Many

of the sex pheromones synthesized by insects are derived from fatty acids as well as some defensive secretions such as quinones, phenols, and carboxylic acids.

Glyceride digestion and absorption results in the appearance of diacylglycerols in the hemolymph. Absorbed fatty acids are incorporated into phospholipids, diacylglycerols, and triglycerols. The predominant lipids in insects are in the form of **triacylglycerols**, the uncharged esters of glycerol (Figure 6.30), and most of this triacylglycerol is located in the fat body where in the cockroach, *Periplaneta americana*, it may make up more than 50% of its wet weight. Triacylglycerols are a concentrated store of energy, with most of the energy coming from the fatty acid component of the molecule. The complete oxidation of carbohydrates and proteins yields about 4 kcal/g, but in contrast, the oxidation of fatty acids yields more than twice this amount, about 9 kcal/g. This is undoubtedly the reason that fatty acids have evolved as the major energy reservoir in animals. As very small animals, insects in particular benefit from the large amount of energy that can be stored in a relatively compact form. Although they are stored in the fat body as triacylglycerides, they are transported in the hemolymph to target cells as diacylglycerides.

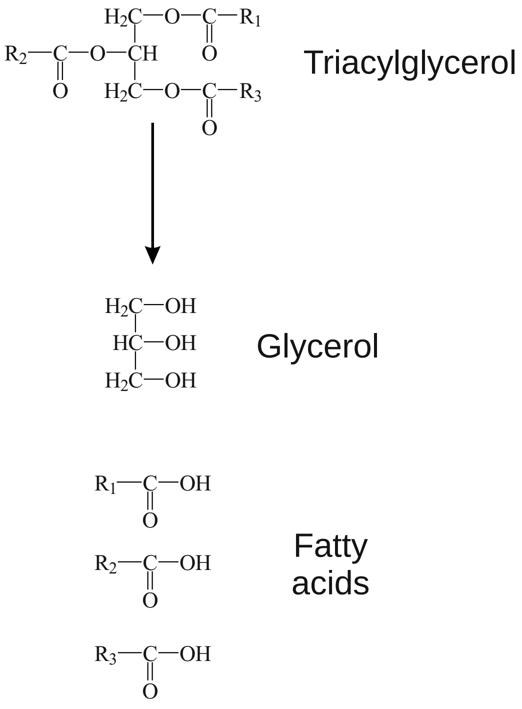


FIGURE 6.30. The breakdown of triacylglycerols into glycerol and fatty acids.

Many of the biochemical pathways involving lipid metabolism in insects are similar to those in vertebrates. Like mammals, most insects are unable to synthesize fatty acids that contain two or more double bonds, and these are therefore required in the diet. Linoleic acid, with two double bonds, is a component of the lipid bilayer of the cell membrane and is an essential fatty acid in vertebrates. Although it was long assumed that insects also must meet their requirement for linoleic acid by its ingestion, a survey of about 35 insect species demonstrated that at least 15 species — including several cockroaches, crickets, aphids, and termites — were indeed able to synthesize linoleic acid from acetate. Of those insects apparently capable of synthesizing all their requirements for fatty acids, the extent to which microorganisms contribute to this ability is not always clear.

In contrast to vertebrates, all insects require a dietary source of sterols because they are unable to synthesize them from precursors, as do most other animals and plants. Sterols are important components of cell membranes, cuticular surface waxes, and precursors for the synthesis of ecdysteroids, the hormones involved in molting. In the cells of most other animals, cholesterol is synthesized from the two-carbon acetate in several sequential steps, but insects lack this ability and must ingest it in the diet. Because most plants do not contain any cholesterol, phytophagous insects must obtain their cholesterol by converting the predominant phytosterols, sitosterol, campesterol, and stigmasterol to cholesterol by the dealkylation of the C-24 alkyl group. In those phytophagous insects that are unable to make this conversion, makisterone A, or 24-methyl 20-hydroxyecdysone, is used as the molting hormone. Makisterone A is thus a 28-carbon ecdysteroid, compared to the 27-carbon skeletons of the other ecdysteroids. The midgut is the major site of cholesterol absorption, but it also may occur through the crop.

Ingested triacylglycerides are hydrolyzed to diacylglycerides and fatty acids by digestive enzymes, and as they enter the hemolymph, they are bound to lipophorins that carry them to target cells. This is another feature that differs from vertebrates, where triacylglycerols are the main lipid. Intracellular lipases break down the acylglycerides into glycerol and fatty acids. Glycerol is phosphorylated to glycerol-3-phosphate and joins the glycolytic pathway. The fatty acids undergo β-oxidation in the mitochondria, which involves the sequential removal of acetyl CoA and two pairs of hydrogen atoms from the fatty acyl CoA and the ensuing generation of $FADH_2$ and NADH. The acetyl CoA is then able to enter the citric acid cycle (Figure 6.31). Before they can cross the inner mitochondrial membrane, however, they must first form an ester with the compound **carnitine**, which facilitates this transport. The production of acetyl CoA from fatty acid oxidation is also a key intermediate for the interconversion of fats, proteins, and carbohydrates.

The capacity of many insects to store polysaccharides tends to be somewhat limited, so the carbohydrate that is ingested above the immediate caloric require-

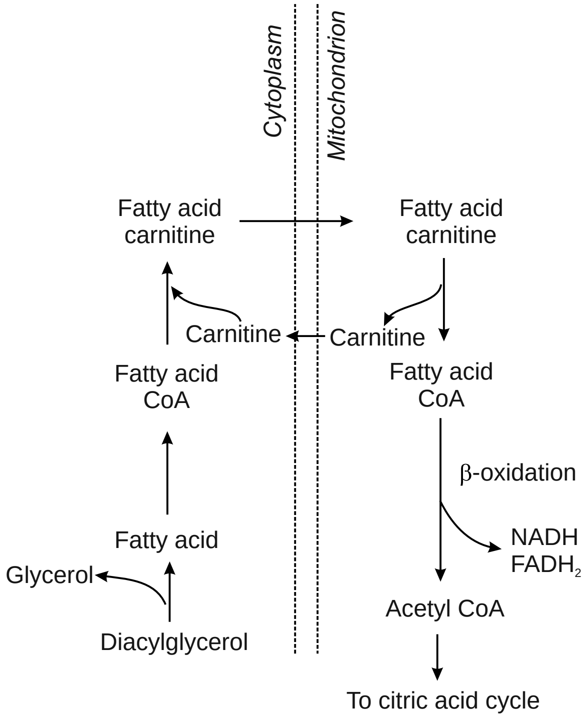


FIGURE 6.31. The entry of fatty acids into the mitochondrion, using carnitine for transport.

ments is often converted to fatty acids that are in turn stored as triacylglycerols in the fat body. The synthesis of fatty acids does not simply occur by a reversal of the same enzymatic steps within the mitochondria that are involved in degradation. Instead, the synthesis of fatty acids occurs in the cytoplasm and is catalyzed by a group of enzymes known as the **fatty acid synthetase complex**. Although the enzyme complex has been isolated from several insects, the details of the synthesis are far from complete, with most of the pathway assumed to be similar to that in vertebrates. There have been differences reported in the mechanisms of fatty acid biosynthesis in the few insects that have been studied, and these differences may be responsible for the variation in the types of fatty acids that are synthesized in each. A single molecule of acetyl CoA serves as a primer, with growth of the chain proceeding by the successive additions of acetyl residues at the carboxyl end. Each acetyl residue is derived from two carbon atoms of malonyl CoA, with the third carbon atom lost as CO_2. The acyl intermediates that form in the process are thioesters of a low-molecular-weight protein, **acyl carrier protein**, or **ACP** (Figure 6.32). A major difference between the fatty acid synthesis in insects and vertebrates is that insects cannot elongate unsaturated

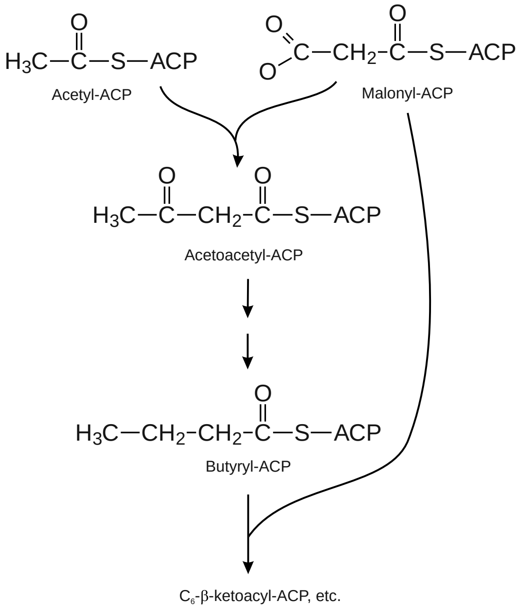


FIGURE 6.32. The synthesis of fatty acids from acetyl-ACP.

fatty acids or introduce additional double bonds into a fatty acid. Another difference is that most animals hydrolyze lipids to free fatty acids that are bound to albumin and transported throughout the animal via the circulatory system. In insects, diacyglycerols are transported bound to lipoprotein instead of free fatty acids bound to albumin.

The site of most lipid synthesis and storage in insects is the fat body, and lipid must be transported from this site to other target cells throughout the body. However, the hemolymph is mostly water, making it difficult for the lipid molecules to dissolve and be carried. A mechanism for the transport of lipids consists of the **lipophorins**, hemolymph lipoproteins that serve as lipid shuttles in the aqueous hemolymph to load and unload a variety of lipid molecules at target sites. The high-density lipophorins (HDLp) have a molecular weight of about 600,000 daltons and contain single molecules of apolipophorin I (apoLp-I) and apolipophorin II (apoLp-II). A third apolipophorin particle, apolipophorin III (apoLp-III), circulates in the hemolymph and is taken up by the HDLp when it acquires diacylglycerols from the fat body. The apoLp-III increases the capacity of the lipophorin to bind the diacylglycerols, and as the ratio of lipid to protein increases with this binding, the molecule becomes less dense and changes the HDLp into a low-density lipophorin (LDLp) (Figure 6.33). The LDLp transfers the diacylglycerols to cells in the flight muscles, ovaries, epidermal cells, and

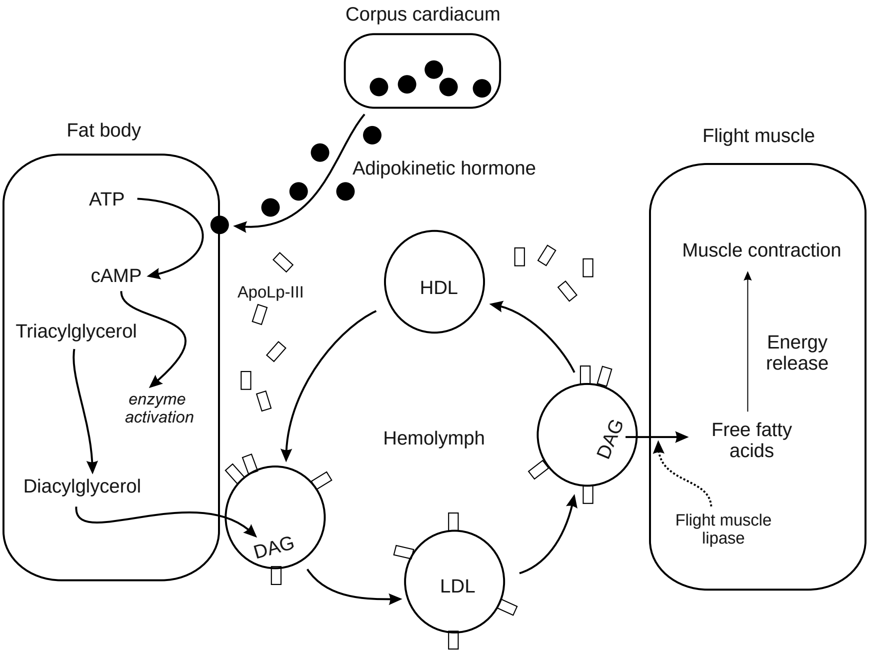


FIGURE 6.33. The transport of lipid from the fat body to flight muscles. Apolipophorin III circulates in the hemolymph and is taken up by the high density lipophorin (HDL) when it acquires the diacylglycerols (DAG) from the fat body. This transforms the HDL into a low-density lipophorin (LDL), which transfers the DAG to the flight muscles.

oenocytes. These target cells unload the diacylglycerols, hydrolyze them, and oxidize the liberated fatty acids for energy. The apoLp-III is released into the hemolymph, and the high-density lipoprotein shuttle molecule is then ready to be loaded once again.

Because lipids are such a compact form of energy storage, they are the storage molecules of choice in migratory insects and those that otherwise engage in prolonged flight. Migratory locusts can fly more than 200 km during flights of 10 h or more. Although their initial energy substrate is carbohydrate, triacylglycerides stored in the fat body begin to be mobilized after 15 to 30 min. The neuropeptide, **adipokinetic hormone** (AKH), is synthesized by the intrinsic neurosecretory cells in the glandular lobe of the corpus cardiacum. Almost 40 different AKH isoforms have been identified from a variety of insect orders with some insects containing multiple forms of the hormone so that each may perform different tasks. The locust *Locusta migratoria* utilizes at least three different AKHs, and the cockroach *Periplaneta americana* has two. The hormones consist of 8 to 10 amino acids with at least two aromatic amino acids. When released into the hemolymph during flight, AKH activates **TAG lipase** in the fat body that

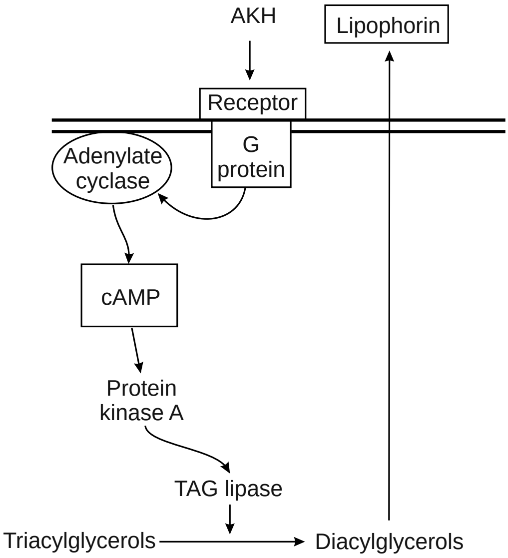


FIGURE 6.34. AKH activates the enzyme TAG lipase that hydrolyzes stored triacylglycerols to diacylglycerols. The diacylglycerols are transported to target tissues by lipophorin.

hydrolyzes the stored triacylglycerides to diacylglercides that are then transported to target tissues by lipophorins (Figure 6.34). The release of AKH is inhibited by blood trehalose and stimulated by octopamine. AKH is also involved in the mobilization of carbohydrate reserves in some insects by its activation of the enzyme glycogen phosphorylase (Figure 6.35) and the increase in hemolymph proline in insects that utilize it as a metabolic substrate for flight.

DIAPAUSE AS A METABOLIC PROCESS

In anticipation of predictable unfavorable environmental conditions under which an insect population would not survive, many species initiate a series of metabolic changes well before the unfavorable conditions actually occur. Unlike migration, which is an escape in space, this **diapause** is an escape in time, allowing the insect to withstand the unfavorable conditions while remaining in place. It is one of many strategies insects use to survive adverse conditions. The term "diapause" was originally applied to a resting stage during embryogenesis and was later used to describe what appeared to be suppressed growth in any stage. However, diapause is not a state but rather an active process or syndrome that advances as the insect progresses through the diapause. In **quiescence**, a state of dormancy is directly imposed by adverse conditions, and the insect recovers

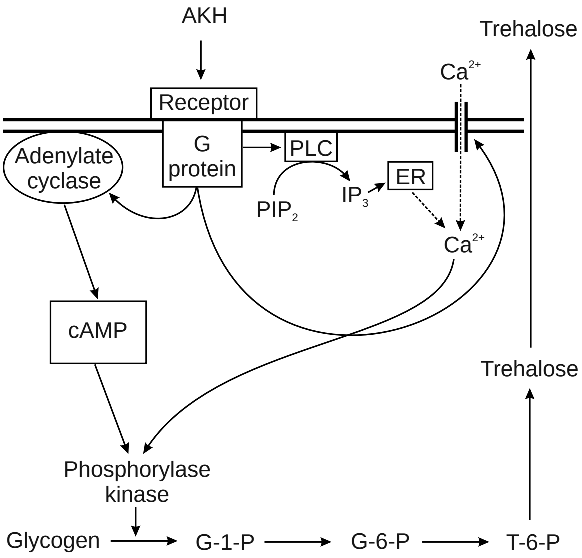


FIGURE 6.35. AKH activates the enzyme phosphorylase kinase that hydrolyzes glycogen to trehalose released into the hemolymph.

to its normal state immediately after the favorable conditions return. Diapause is profoundly different from quiescence in that it is brought about by conditions such as a critical photoperiod that themselves are not adverse, and it involves an alteration of the hormonal conditions during species-specific periods of the life cycle. Insects enter diapause when environmental conditions are still favorable for development. In some insects, an **obligatory diapause** occurs at a specific stage in each generation and is independent of environmental conditions, resulting in univoltine populations. However, most insects undergo a **facultative diapause** and can choose between entering diapause or continuing direct development based on the environmental stimuli that a particular generation is exposed to.

Diapause can be divided into three main phases. Insects have evolved the ability to assess the predictable progression of seasons from the cues of day length, and during **prediapause**, there is a sensitivity to these changes in photoperiod, most commonly the change from long to short days. Tropical insects can use cycles of temperature to activate a diapause that allows them to escape high temperatures and drought conditions. The way that the insect counts days and daylight hours per day is not understood but involves the PER protein encoded by the gene *per*, the only gene so far implicated in prediapause development. The mechanism governing circadian behavior by the PER protein is more completely outlined in Chapter 5. Prediapause may include a preparative stage

that is characterized by behaviors that bring the insect to a special overwintering site for better protection or by active behaviors that modify the environment to escape extreme conditions. Caterpillar larvae can cause the leaf they feed on to roll up, forming a protective enclosure that may trap solar heat and protect the insect from predation during the later diapausing stage. Substantial metabolic changes may occur during the preparative stage of diapause, with increases in the production of proteins used to store nutrients and to prevent ice formation in the hemolymph.

During the **diapause stage**, normal morphogenesis is channeled into an alternative physiological program. Although the usual developmental programs are blocked, metabolic activity continues in support of these alternative pathways. Free living adults may continue to eat, but they convert the food into energy reserves and continue to produce the components that contribute to cold-hardening. The synthesis of diapause-associated proteins by the fat body that began during prediapause continues during diapause. The diapause syndrome generally does not require a specific stimulus for its termination. Insects with an overwintering diapause end the syndrome during midwinter but do not begin development until the spring. Toward the end of the diapause stage, the insect once again becomes capable of initiating development, but low temperatures may prevent it from responding. The **postdiapause stage** is reached when the insect is capable of initiating development once favorable conditions return.

Diapause is initiated by environmental signals but is regulated by hormones. Embryonic diapause may be controlled by elevated titers of ecdysone, or in the best known system in the silkworm moth, *Bombyx mori*, there is a maternal determination of whether an egg diapause occurs in the next generation by the production of **diapause hormone** (DH). DH is produced by the generation of female adults that is exposed to the long photoperiod of midsummer days and promotes diapause in developing eggs that would otherwise hatch and begin developing during the unfavorable autumn and winter months. In effect, the embryo is relying on its mother to make a decision to diapause.

When larvae are exposed to a short photoperiod, those adults later produce the 24 amino acid DH from their subesophageal ganglion that is incorporated into the eggs and causes the resulting embryos to halt their development at gastrulation. By stimulating the production of the enzyme trehalase in the ovaries, DH regulates the accumulation of glycogen in the developing ovary and determines the metabolic fate of the glycogen that is deposited in the egg. In non-diapausing eggs, glycogen levels remain high, but in diapausing eggs, levels of glycogen decrease significantly at the same time that the polyols sorbitol and glycerol increase. Sorbitol not only may provide a degree of freeze-tolerance as a cryoprotectant, but it also directly inhibits further embryonic development. The stimulus for the termination of embryonic diapause is prolonged chilling that allows the sorbitol and glycerol to be reconverted to glycogen, which eliminates the inhibition by and provides an energy source for postdiapause

development and hatching. This mechanism involving DH has only been reported to occur in *Bombyx*.

Bombyx eggs that enter diapause also contain maternal ecdysteroids conjugated as phosphoric esters, but these are hydrolyzed to the free forms of active ecdysteroids in nondiapausing eggs. Levels of free 20-hydroxyecdysone increase significantly in eggs that will develop, but they remain low in diapause eggs. Because of the strong correlation between 20-hydroxyecdysone and embryonic molting, these increases in free ecdysteroids may also be involved in the continuation of embryonic development.

Ecdysone is generally involved in larval and pupal diapause, either as a result of the failure of the brain to release PTTH or of the prothoracic gland to respond to PTTH until it has experienced an adequate chilling. Some lepidopterans enter a larval diapause characterized by stationary molts resulting from elevated JH. Adults commonly undergo a reproductive diapause mediated by the absence of JH.

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Circulatory Systems

All cells need to exchange materials with their environments. To function properly, cells must take up nutrients, discard metabolic wastes, and receive chemical messages from hormones. These substances can pass through the cell membrane only if they are dissolved in water, and for this reason, every living cell must be surrounded by an aqueous medium. In single-celled animals, this exchange of materials occurs by simple diffusion or active transport through the cell membrane, because the large ratio of surface area to volume in these small organisms provides an area sufficient for the exchange of materials. In larger multicellular animals, including humans and other vertebrates, diffusion alone could not accomplish this exchange. Nutrients that are absorbed through the digestive tract would never reach cells in fingers or toes if they had to travel through the body cavity by diffusion. In vertebrates, a closed system of internal transport consisting of the heart, blood vessels, and the enclosed blood is necessary to bathe cells and transport materials to and from them throughout the body.

Although insects are also multicellular animals, they are small enough to allow diffusion to serve as a mechanism of metabolic exchange. The extensive tracheal system in insects carries oxygen to the cells, and, with the exception of the few species that supplement oxygen transport with hemoglobin, the circulatory system has little function in oxygen transport (see Chapter 9, Respiratory

Systems). As a medium that is used primarily for the transport of chemical agents, its role is far less demanding than for vertebrate blood that has the absolute requirement for oxygen transport. The most important roles of insect hemolymph are to serve as a medium that bathes cells and transfers substances to and from them, as a reservoir of water and metabolic substances, as a medium for cellular and humoral defense, and, in soft-bodied insects, to provide the necessary hydrostatic pressure for molting and maintenance of body shape. It also may protect cells from freezing, contain substances that deter predation, and provide a medium for the retention of metabolic heat that allows the insect to remain active at low environmental temperatures.

STRUCTURE OF THE INSECT CIRCULATORY SYSTEM

The body cavity of insects consists of a series of sinuses that are collectively referred to as the **hemocele**. The only closed portion of the circulatory system is the **dorsal vessel**, a tube that extends the length of the body from the posterior end of the abdomen into the head (Figures 7.1A and B). Within the head, it passes under the brain just above the digestive tract, and then opens anteriorly. The dorsal vessel is not a uniform tube but consists of two segments, the **heart** and **aorta**, which are formed during embryogenesis from the **cardioblasts** of the embryonic mesoderm.

The segment of the dorsal vessel that is known as the heart makes up the posterior portion that is found mainly within the abdomen. The heart is composed of a series of segments or chambers that contain paired lateral openings called **ostia** (Figure 7.2). Incurrent ostia are simple valves that allow hemolymph

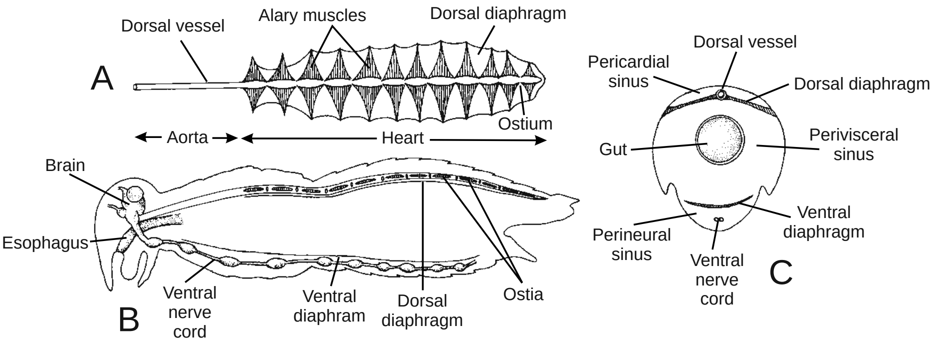

FIGURE 7.1. The generalized insect circulatory system. A. Dorsal view of the heart and aorta. B. Longitudinal section of the insect body, showing the dorsal and ventral diaphragms. C. Cross section through the abdomen, showing the sinuses created by the diaphragms. From Romoser and Stoffolano (1998). Reprinted with permission.

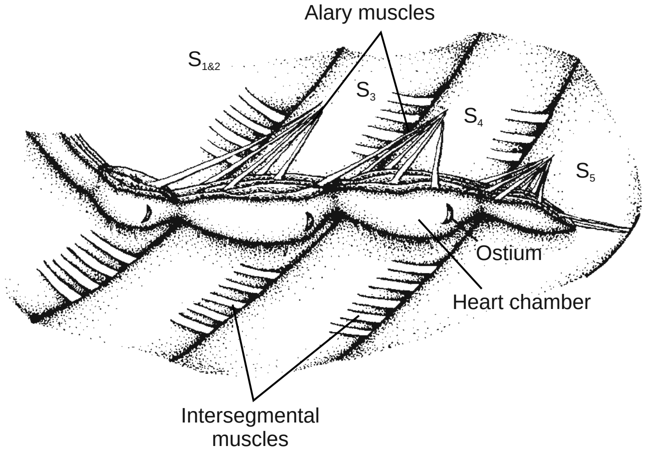

FIGURE 7.2. The ostia and alary muscles of the heart of *Stomoxys calcitrans*. From Cook and Meola (1983). Reprinted with permission.

to enter the lumen of the heart from the pericardial sinus at diastole but prevent its outflow during systole directly into the body cavity. Excurrent ostia have no valves and sometimes open caudally from the posterior of the heart in some Diptera or laterally in the more primitive Thysanura, Orthoptera, and Plecoptera. In a few insects, internal valves are present that prevent the backward flow of hemolymph between the chambers within the heart as it pumps the hemolymph forward to the head. These are generally rare, however, although the appearance of the ostia in many insects often suggests the presence of valved chambers. The heart can be wrapped with both circular and longitudinal muscles, some of which may be more prevalent in anterior or posterior chambers.

The heart is supported in the hemocele by connective tissue strands and from 2 to 12 pairs of fanned-shaped **alary muscles** that, along with connective tissue, form the dorsal diaphragm that lies just below the heart. The diaphragm extends through the abdomen and creates a **pericardial sinus** in the compartment that surrounds the dorsal vessel (Figure 7.1C). Large **pericardial cells** are usually located on the dorsal diaphragm and function as phagocytic organs to filter out large particles from the hemolymph. In some insects, segmental blood vessels may also extend laterally from the heart (Figure 7.3).

The aorta, the anterior portion of the dorsal vessel, extends from the thorax into the head. It is a simple unbranched tube that is thinner than the heart and lacks ostia. It is often attached to the brain and pharynx by connective tissue, passing underneath the brain and opening behind the pharynx. In most adult Lepidoptera and Hymenoptera, the aorta loops through the thoracic flight muscles before it enters the head. In some higher Diptera, a cephalic pulsatile organ is also present in the posterior region of the head that facilitates the distribution of hemolymph into either the head or the thorax.

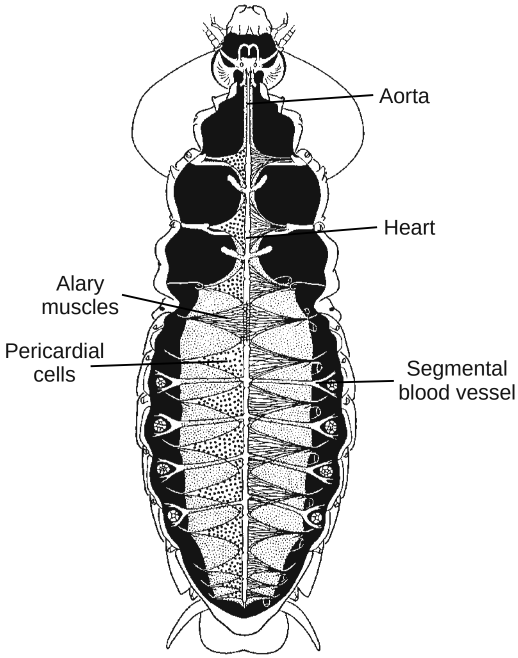

FIGURE 7.3. Dorsal view of the circulatory system and the segmental blood vessels that extend laterally from the heart. From Nutting (1951). A comparative anatomical study of the heart and accessory structures of the Orthopteroid insects. *Journal of Morphology* 89: 501–598. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

A more advanced feature that further compartmentalizes the hemocele is a **ventral diaphragm**, a structure generally absent in the most primitive insect orders. This diaphragm is located just above the ventral nerve cord in the abdomen and divides the hemocele into a **perineural sinus** (Figure 7.1C), with the remaining portion of the hemocele that surrounds the gut termed the **perivisceral sinus**. Along with the dorsal diaphragm, undulations of the ventral diaphragm can control the distribution of hemolymph within the compartments of the hemocele. Higher dipterans lack a ventral diaphragm but have large tracheal air sacs that partition the hemocele so that a smaller volume of hemolymph can function more economically when a reduced weight is required for flight.

Accessory Pulsatile Organs

One problem with an open circulatory system is that without a directional flow of hemolymph, outlying dead-end structures such as legs, antennae, cerci, and

wings have difficulty receiving the circulating fluid. The dorsal vessel pumps hemolymph only to the head where it then must diffuse backward passively through the hemocele, and diffusion alone is insufficient to provide the distant cells of these appendages with nutrients. In particular need for hemolymph exchange are the antennal sensory receptors that must respond quickly to changes in environmental signals. Dealing with this problem are special **accessory pulsatile organs** at the base of each of these structures to channel the hemolymph into them. A large number of these accessory pulsatile organs may be present and are completely separate from the functioning of the dorsal vessel (Figure 7.4).

Accessory pulsatile organs at the bases of the legs pump hemolymph into a ventral sinus and out of a dorsal sinus created by an internal septum that divides them internally to maintain a directional flow (Figure 7.5). They have thus far only been found in hemipterans and orthopterans. In locusts, the mesothoracic legs contain a diaphragm between the trochanter and femur that is moved by muscles, and in coordination with changes in the size of tracheal air sacs during respiration, hemolymph is pumped into the leg sinus that is created and divided into channels by a septum (Figure 7.6). The pumping activities of these organs

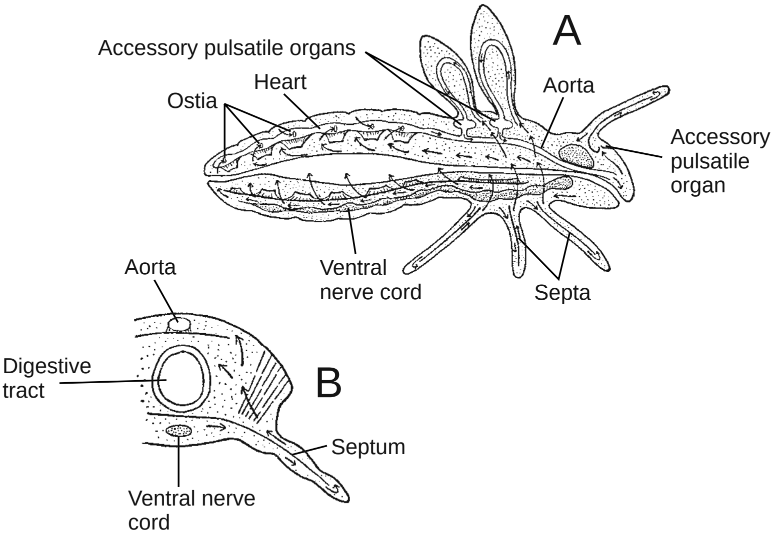

FIGURE 7.4. The movement of hemolymph through the circulatory system of a generalized insect. A. Arrows show the movement of hemolymph forward through the dorsal vessel and backward through the Hemocele, aided by accessory pulsatile organs at the bases of appendages. Dorsal and ventral diaphragms and septa in appendages create a directional flow. B. A cross section through the thorax showing the circulation around the leg septum and diaphragms. From Wigglesworth (1974). Reprinted with permission.

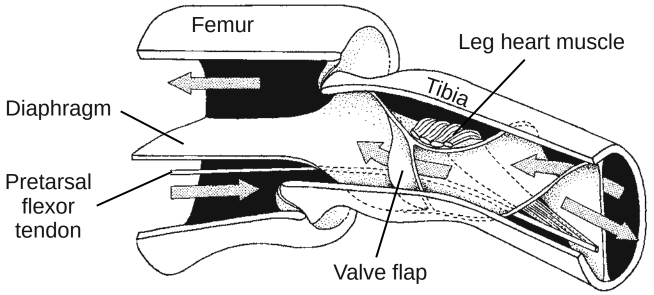

FIGURE 7.5. An accessory pulsatile organ in the insect leg. Arrows show the direction of hemolymph flow. From Hantschk (1991). Reprinted with permission.

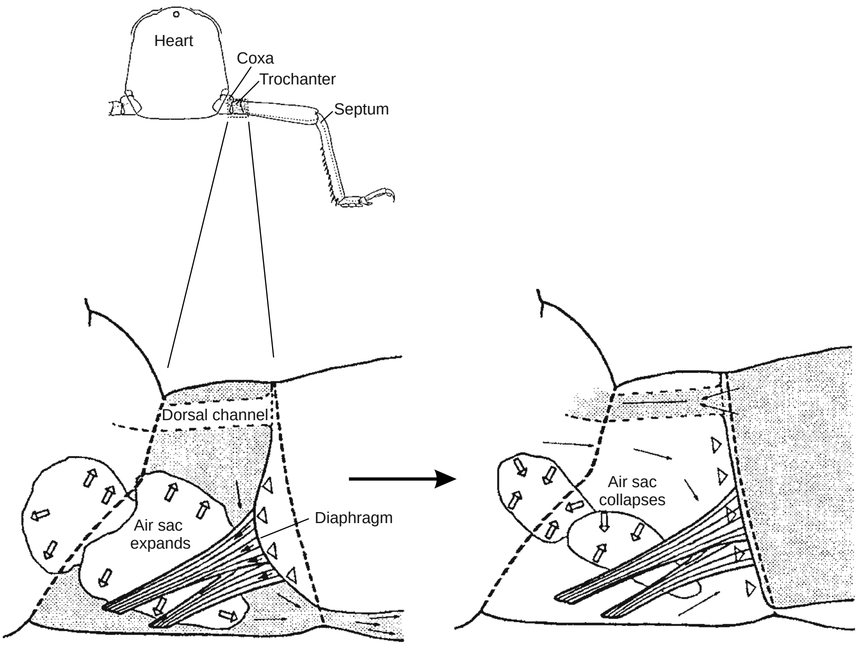

FIGURE 7.6. Tracheal air sacs can regulate the movement of hemolymph into the leg. From Hustert (1999).

are independent of those of the dorsal vessel and each other, often beating faster than the rate of the heart.

Although most of the epidermal cells of the wing have degenerated to form the light-weight wing membrane, hemolymph flow is necessary within the wings to maintain the nerves and sensory receptors and aid in thermoregulation.

Movement of hemolymph through the wings occurs as a result of accessory pulsatile organs at the wing bases, found so far in all species with wings. These may be swellings of the dorsal vessel or aortic diverticulae with incurrent ostia (Figure 7.7A). In some insects, paired or unpaired pulsatile diaphragms not associated with the dorsal vessel may be present (Figure 7.7B and C). There may be an actual flow of hemolymph through the wings or a one-directional flow occurring in all veins simultaneously that is periodically reversed. These accessory organs generally create a negative pressure in the body cavity that sucks hemolymph out of the wings with relaxation of the organs allowing an inward flow. Pulsating areas within the wing veins themselves that are distinct from the thoracic organs may also be present.

Pulsatile organs in the antennae consist of a noncontractile sac connected to a long blood vessel that reaches to the tip of the antenna. More advanced insects retain a muscle that compresses the sacs and pumps hemolymph through the antenna (Figure 7.8). The ancestral condition, found in the Entognatha, is a direct connection between the antennal vessels and the dorsal vessel. In Diplura, extensions of the aorta reach into the antennae and cerci (Figure 7.9). The

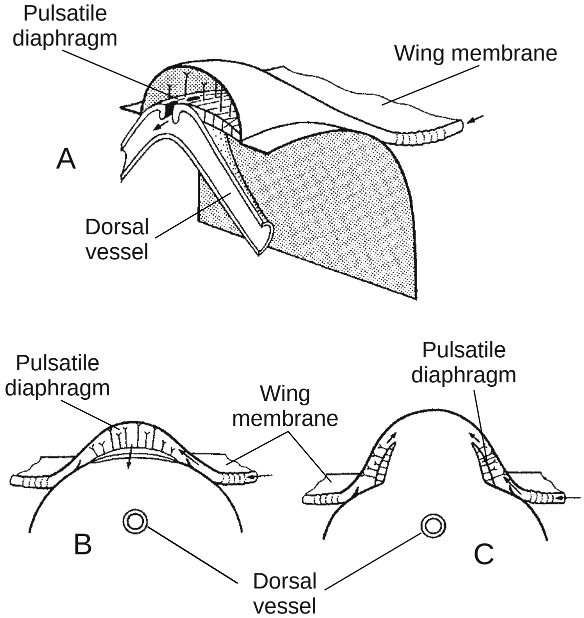

FIGURE 7.7. Accessory pulsatile organs associated with the wings. From Pass (1998). Reprinted with permission. A. From the enlargement of the dorsal vessel. B, C. Not associated with the dorsal vessel.

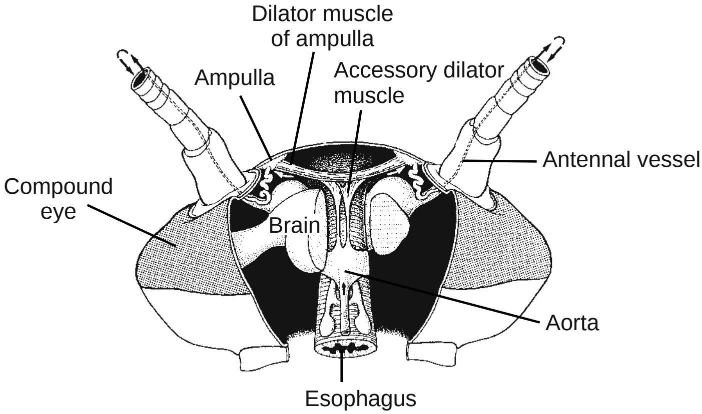

FIGURE 7.8. An ampulla and its dilator muscles that pump hemolymph into the antennae. From Pass (1985). Reprinted with permission.

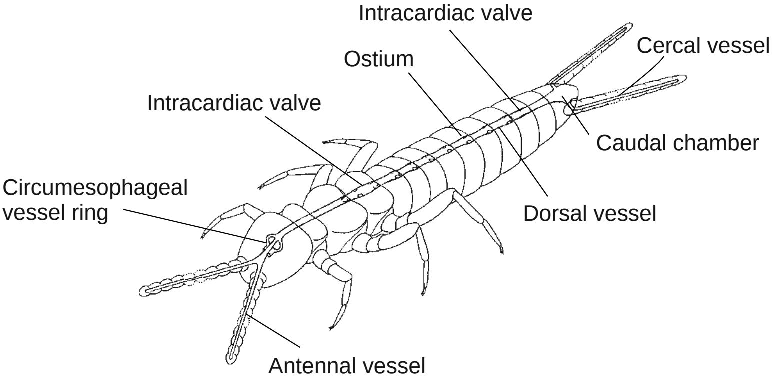

FIGURE 7.9. The extension of the circulatory system into the antennae of diplurans. From Gereben-Krenn and Pass (1999). Reprinted with permission.

evolution of the ectognathous head capsule may have resulted in a reconstruction that caused the antennal and dorsal vessels to separate. The contractions of these accessory pulsatile organs are usually myogenic and originate in the muscles themselves, but they may be modulated by the nervous system. In *Periplaneta*, the antennal pulsatile organs are innervated by the subesophageal ganglion and contain neurosecretory terminals.

Heartbeat and Its Regulation

The dilation of the chambers of the heart by the alary muscles causes hemolymph to enter through the incurrent ostia. The ostia close as the muscles that lie within its wall cause the chambers of the heart to contract. The wave of peristaltic contractions moves forward along the entire length of the heart, pushing the hemolymph forward that had entered the heart though the ostia during relaxation into successive anterior chambers. The rate of heart contraction varies between species and between stages of development within a species, making it impossible to generalize the rate throughout the insect orders. Environmental factors, such as temperature, affect heartbeat, with higher temperatures causing increased rates. At extreme high and low temperatures, the heartbeat may stop entirely. It is not uncommon, especially in pupae, for the heart to stop beating for several seconds and even to reverse its contraction so that peristaltic waves move from the front to the back. These backward contractions may result when the pressure in the anterior of the insect is high or when excurrent ostia become blocked by the histolyzed tissues in the pupa. The heart of the adult blowfly, *Calliphora vomitoria*, has been observed to beat forward as fast as 376 beats per minute and backward at about 175 beats per minute. Although the aorta may show rhythmic pulsations from hemolymph being pumped through it, it does not itself beat.

Like the accessory pulsatile organs, the contractions of the heart are regulated myogenically but can be influenced by nervous and endocrine stimuli. In many insects, such as larval *Drosophila,* the dorsal vessel has no innervation and the heartbeat is entirely myogenic. In others, the aorta is innervated by a median nerve from the hypocerebral ganglion of the stomatogastric nervous system. The heart of adult *Drosophila* becomes innervated during metamorphosis and is supplied with neurons that use glutamic acid as a neurotransmitter at the neuromuscular junction. These neurons innervate the longitudinal muscles that initiate the reverse heartbeat at certain times. Segmental nerves arising from the ventral nerve cord may also innervate the alary muscles and the heart. The alary muscles are well innervated and may contribute to the heartbeat, especially during diastole.

Cardioacceleratory peptides (CAPs) that are produced by the corpora cardiaca and ventral nerve cords may modulate heartbeat. In *Manduca sexta*, CAPs stimulate the heart to contract immediately after adult emergence and during flight. This increase in hemolymph pumping after adult emergence allows the wings to inflate. During flight, the increased heartbeat rate facilitates hemolymph transfer between the abdomen and thorax so the flight muscles do not overheat with activity. CAP axons also innervate the hindgut and may be responsible for hindgut myotropic activity. CAP causes an increase in hindgut contractions that are associated with the gut purging in *Manduca* larvae that occurs during their wandering behavior just before pupation. The **crustacean cardioacceleratory**

peptide (CCAP), which was first identified as CAP2a in *Manduca*, has been shown to be identical to the crustacean peptide that triggers ecdysial behavior in the crab and is also present in lobsters and mollusks. CCAP has a wide range of effects in *Drosophila*, *Manduca*, and *Locusta*, increasing the rate of the heartbeat, stimulating gut contractions, and acting on the Malpighian tubules to increase fluid secretion. It also triggers ecdysis during the larval-pupal molt of *Manduca.*

Neurosecretory cells may be contained within the aorta or fused with its walls. Neurosecretory axons arise from cell bodies in the brain and subesophageal ganglion and often terminate on the aorta wall where they may form a neurohemal organ. CCAP neurons in the CNS of *Manduca* are found in the brain, subesophageal ganglion, and abdominal ganglia, expressing the single CCAP gene in this insect. The heart may also be secretory itself. The hearts of the lepidopteran *Calpodes* and the hemipteran *Rhodnius* synthesize peptides of unknown function that may be released into the hemolymph.

Hemolymph Composition

The hemolymph is the major extracellular fluid in insects. It makes up from 15% to 75% of the volume of the insect, varying significantly with species and individual physiological state. The hemolymph is the major transport medium for the exchange of materials between cells, such as hormones, waste materials, and nutrients. Through its regulation of ionic and chemical composition, it maintains the proper internal environment for cells as an extracellular extension of intracellular fluids. In this role, it contributes to the ability of the insect to live at both high and low temperatures. It serves as a major compartment and storage reserve for water. The hemolymph is far from being a static reservoir for the storage of metabolites, however. It is a dynamic tissue that changes with the changing physiological state of the insect. Additionally, it maintains the hydrostatic pressure required to maintain body shape in soft-bodied insects and to facilitate the splitting of the cast skin at ecdysis. The volume of the hemolymph often increases toward the end of each stadium and contributes to the rupture of the old cuticle. The **ptilinum** of flies is inflated by hemolymph to allow the adult to exit from the hardened puparium (Figure 7.10).

The hemolymph consists of the liquid **plasma** and the cellular **hemocytes**. Plasma composition is variable. It is usually clear but may be colored green or yellow in some insects, reflecting the pigments that are present. Its pH can also be variable; it is generally slightly acidic but may be alkaline in some species. A variety of soluble components contribute to its total osmotic pressure. In most other animals, the major inorganic components of the body fluid are sodium and chloride, but in insect blood the composition can be quite different. In primitive apterygotes, sodium and chloride do indeed appear to be the most important osmotic effectors. Primitive exopterygotes, including Ephemeroptera,

FIGURE 7.10. The ptilinum of dipterans that is inflated at adult emergence and used to escape from the puparium. From Jones (1977). Reprinted with permission.

Odonata, and Dictyoptera, also largely use sodium and chloride, but with contributions from magnesium, potassium, and calcium. In endopterygotes, such as Diptera, Mecoptera, and Neuroptera, sodium is again an important cation, but chloride is replaced by higher concentrations of amino acids and other organic components (Figure 7.11). In the endopterygotes Hymenoptera and Lepidoptera, amino acids and other organic molecules play a major role along with potassium but with a reduced involvement of sodium. These ionic differences were once attributed to either phytophagous or carnivorous diets, because plant-feeding insects contained higher levels of potassium and insects feeding on other diets had higher levels of sodium. However, this generalization has numerous contradictions, and the relationship between the ionic composition of the hemolymph and basic diet of the insect is not entirely clear.

The concentration of free amino acids in the insect hemolymph can be as high as 50 to 100 times that of mammalian plasma. For example, the total hemolymph amino acids reach 150 mmol/L in tsetse compared to about 2 mmol/L in humans. As discussed previously, these amino acids become more prominent in evolutionarily advanced groups. Exopterygotes are characterized by lower levels of amino acids than in endopterygotes and show uniform concentrations of the amino acids that are present. In contrast, endopterygotes contain some amino

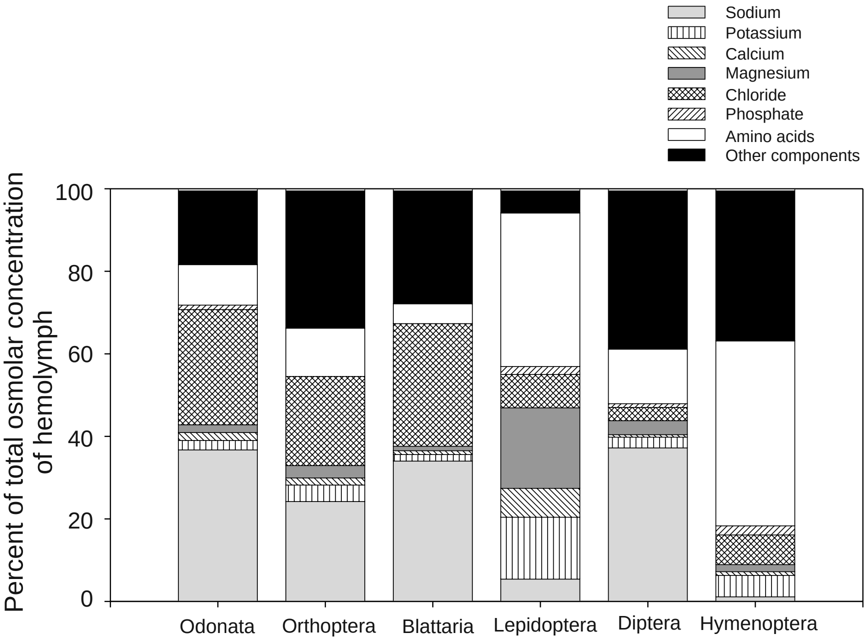

FIGURE 7.11. Components of the hemolymph in representatives of six insect orders. Adapted from Sutcliffe (1963).

acids in much higher concentrations than others. For example, glutamic acid and proline are often found in significantly higher concentrations in the amino acid pool depending on the physiological state of the particular insect. Levels of free amino acids tend to be higher in females than in males, especially during egg maturation. In several insects, proline is used as a substrate for flight and declines during activity as alanine concentrations increase. Proline is also the predominant amino acid in honey bee queens, workers, and drones. The concentrations of methionine, glutamic acid, and aspartic acid are correlated with the activity of the silk glands during the development of the silkworm moth. Leucine and isoleucine tend to be present in lower concentrations in most insects.

Other organic components of the hemolymph include carbohydrates, various Krebs cycle intermediates, uric acid, and soluble proteins. As discussed in Chapter 6, trehalose, an α-1,1 disaccharide of two glucose residues, serves as the major circulating energy source in most insects (see Figure 6.22). It also is used as a cryoprotectant that stabilizes cell membranes to prevent damage from freezing and plays a role in the regulation of feeding. It is present at concentrations of 5 to 50 times higher than glucose, the circulating energy source in vertebrates. This higher level in insects may be one of the compromises necessary to make up for the inefficiency of the circulatory system in distributing these materials

by diffusion. The blood sugar in insects must exist in a higher concentration to ensure that it reaches remote areas of the hemocele in sufficient concentration. If glucose were to serve this function as it does in vertebrates, the higher concentration required in the blood would interfere with the uptake of glucose by diffusion through the digestive tract. By using the disaccharide trehalose in the blood, which is usually rare in the diet, high levels of blood sugar can be maintained without interfering with the uptake of glucose. Levels in the hemolymph do not appear to be regulated homeostatically, however, as they can vary significantly depending on physiological state.

Soluble proteins in the hemolymph include the vitellogenins, which are yolk proteins produced by the female fat body and taken up by the oocytes. Several enzymes, including esterases, chitinases, and proteases, appear in the hemolymph depending on the developmental stage of the insect.

The hemolymph may be a physical deterrent to predation. **Autohemorrhaging**, or **reflexive bleeding**, occurs in some insects when they are attacked. Hemolymph that is fortified with defensive terpenoids such as cantharadin may be released outside the body through intersegmental membranes to discourage ants and other insect predators. The loss of hemolymph may be substantial; in chrysomelid beetles, as much as 13% of the wet weight of the larvae can be lost through autohemorrhage. The hemolymph clots immediately and can bind ants in the coagulum that is formed. In many cases, the hemolymph is returned to the body cavity and the wound heals rapidly.

Hemocytes

The cellular hemocytes are suspended in the plasma but may often remain attached to other body tissues rather than circulate with the blood. They originate from embryonic mesodermal tissue and differentiate during embryogenesis into several distinct types. They have a variety of functions, including phagocytosis of foreign particulate matter, encapsulation of multicellular parasites, and coagulation and wound healing after injury, and they also play an important role in metabolism.

The descriptions of the types of hemocytes present in insects have been varied, perhaps reflecting the procedures used to study them and the differences that may exist between species. For example, hemocytes of different morphologies may be reported when they are observed either through transparent wing veins or in fixed blood smears. Hemocytes are generally classified according to their size, shape, nuclear characteristics, cytoplasmic inclusions, and presumed function. The hemocytes of relatively few species have been examined, and for the most part their functions have been inferred from their morphology. Genetic markers have been most recently employed to differentiate the various hemocyte types.

Several types of hemocytes have been reported most frequently (Figure 7.12). They are continually produced during the larval stages from dividing stem cells and from the continued division of those hemocytes already in circulation in adults. Total hemocyte counts in adult *Schistocerca* locusts range from 7000 to 8000 per microliter of hemolymph.

Prohemocytes are the small, round cells that contain a large nucleus and do not engage in phagocytosis. These cells are believed to be the stem cells that postembryonically give rise to other types. **Plasmatocytes** are larger, more ameboid, pleiomorphic cells with a nucleus surrounded by large amounts of cytoplasm. Plasmatocytes are usually among the most abundant of the hemocytes and are frequently engaged in phagocytosis. **Granulocytes** are compact cells with a small nucleus surrounded by a large cytoplasm with abundant granules and may differentiate into the remaining granulocyte types. **Adipohemocytes** are round cells containing a small nucleus surrounded by a large amount of cytoplasm that contains a number of lipid vacuoles. **Spherule cells** are nonmotile and also have large inclusions that may obscure the appearance of the small nucleus. **Oenocytoids** are ovoid and variable in size with a small nucleus and

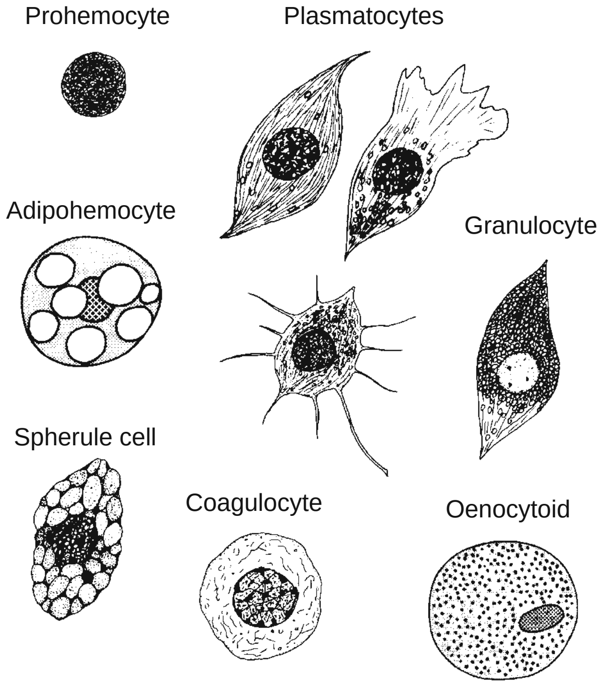

FIGURE 7.12. The generalized morphology of some typical hemocytes that are found in insect hemolymph. From Woodring (1985). Reprinted with permission.

a large complex cytoplasm. They are also nonmotile. **Cystocytes** are fragile cells that rapidly degenerate upon fixation. Fixed cells are ovoid with a small nucleus and granular inclusions within the cytoplasm. A possible hemocyte pathway is shown in Figure 7.13, with prohemocytes able to differentiate directly into plasmatocytes, granulocytes, or spherule cells.

A slightly different terminology has been adopted to describe the hemocytes in *Drosophila.* In addition to the prohemocyte stem cells, **macrophages**, a form of plasmatocyte, are present in *Drosophila* embryos. In the larval stages, plasmatocytes and two additional cell types, **lamellocytes** and **crystal cells**, have been reported. Lamellocytes have only been observed in larvae and appear in massive numbers after an immune challenge. They appear to be phagocytic like the plasmatocytes and can also isolate invading cells by encapsulation. Eggs that are commonly laid in larval dipterans by hymenopterous parasites are encapsulated and walled off by lamellocytes. The crystal cells are more similar to the oenocytoids of other insects and are unable to adhere to foreign surfaces. They are present in low numbers of less than 50 per larva, increasing slightly during the latter part of the third instar and then declining again. Plasmatocytes are the only hemocytes in adult *Drosophila.* Their numbers range between 1000 to 2000 per adult insect.

Hemocytes may be produced in **hemopoietic organs**, permanent aggregations of hemocytes that give rise to more hemocytes by mitosis. They may be localized near the heart and alary muscles. A larval *Drosophila* hematopoietic organ, the **lymph gland**, differentiates from mesodermal tissue toward the end of embryogenesis. It consists of four to six paired lobes located along the dorsal

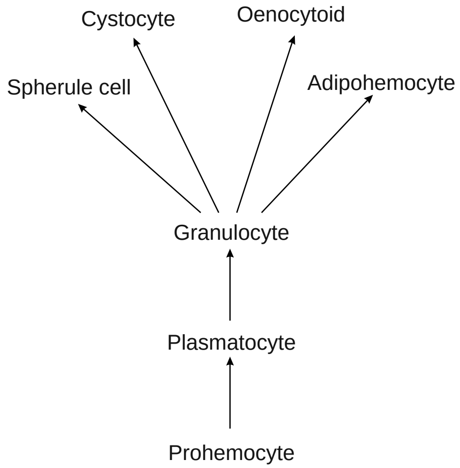

FIGURE 7.13. A possible scheme of hemocyte differentiation. From Gupta (1985). Reprinted with permission.

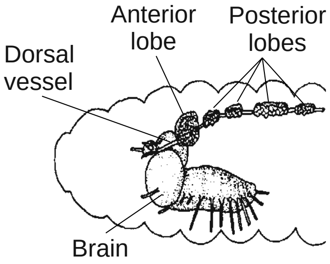

FIGURE 7.14. The anterior and posterior lobes of the larval *Drosophila* lymph gland that produces larval hemocytes. From Sorrentino et al. (2002). Reprinted with permission.

vessel and is responsible for the production of larval hemocytes (Figure 7.14). The anterior and posterior lobes of the lymph gland contain plasmatocytes that appear to be the same as those in circulation, in addition to crystal cells and prohemocytes. The plasmatocytes disperse throughout the embryo and are responsible for the phagocytosis of apoptotic cells, and the crystal cells participate in the melanization of invading pathogens. Crystal cells contain a prophenol oxidase and, when lysed, release the active enzyme that catalyzes the synthesis of melanin. The lymph glands disappear during the pupal stage and adult hemocytes are carried over from those produced during the larval stage. No adult hematopoietic organ has been identified, and adult hemocytes do not undergo cell division.

Like all other insect cells, hemocytes require a source of oxygen for respiration. However, unlike other insect cells, they are suspended in the hemolymph and thus have difficulty deriving their oxygen directly from the tracheal system. In the larvae of *Calpodes*, tufts of special aerating trachea fill a compartment, the **tokus**, at the tip of the abdomen near the heart (Figure 7.15). Hemocytes migrate to the tokus where they become aerated and are then circulated through the dorsal vessel. This modification of the tracheal system in the tokus thus serves as a lung for the oxygenation of hemocytes.

IMMUNE MECHANISMS IN INSECTS

Most insects live in close proximity to microorganisms that could easily establish infections if lines of defense were not established. Although they are vectors of a number of microorganisms that can be retained and nourished, these parasites are kept in check and remain within only certain tissues before being transmitted. Insects must also counter the infestations of multicellular parasites, including fungi and the eggs of insect parasitoids. To deal with these infections, insects have evolved a relatively sophisticated system of biological defense (Figure 7.16).

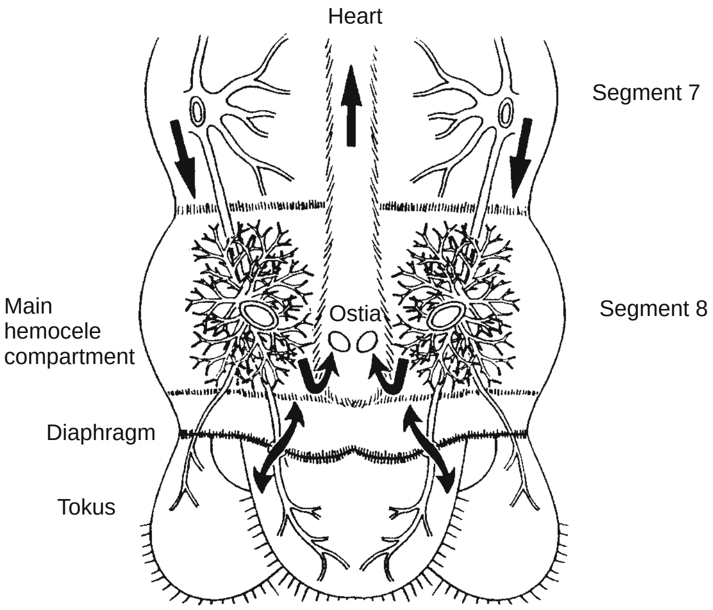

FIGURE 7.15. The tokus, a structure at the tip of the abdomen of larval *Calpodes*, which oxygenates hemocytes. From Locke (1998). Reprinted with permission.

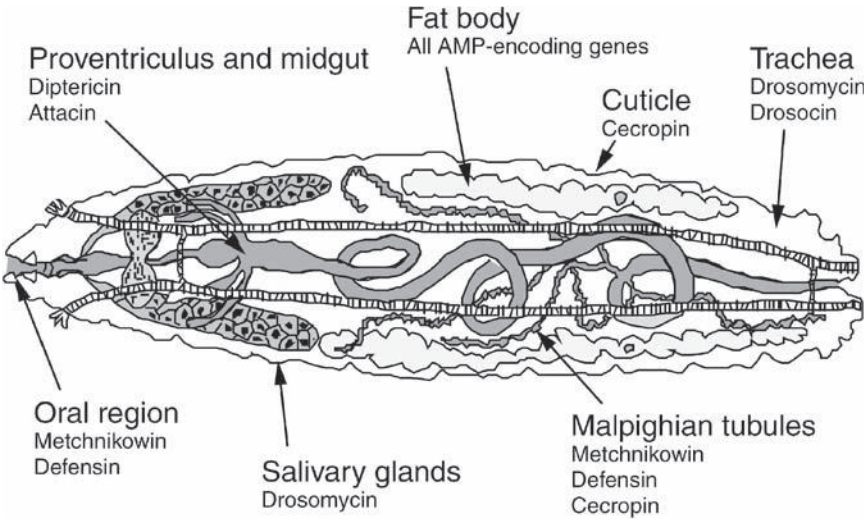

FIGURE 7.16. Biological defense barriers to infection in *Drosophila* larvae. From Tzou et al. (2002). Reprinted with permission.

When *Drosophila* larvae are injected with *E. coli*, it takes only 6 h to clear the hemolymph of the infection.

The primary barriers to infection are the outer cuticle and the digestive tract that are in direct contact with the environment and are able to physically exclude potential parasites. These barrier epithelia, including tracheae, Malpighian tubules, and cells of the reproductive tract, may be induced to express antifungal and antibacterial peptides upon infection or abrasion (Figure 7.17), similar to the production of antimicrobial peptides in the lungs, reproductive tracts, digestive tracts, and saliva by vertebrate epithelia.

Breaches in the cuticle cause an immediate clotting of the hemolymph and melanization at the site of injury. The clots prevent further loss of body fluid, especially important in soft-bodied larvae that depend on hemolymph pressure to maintain their hydroskeletons, and also restrict the infection of microorganisms. With an open circulatory system, arthropods are able to rely on a clotting

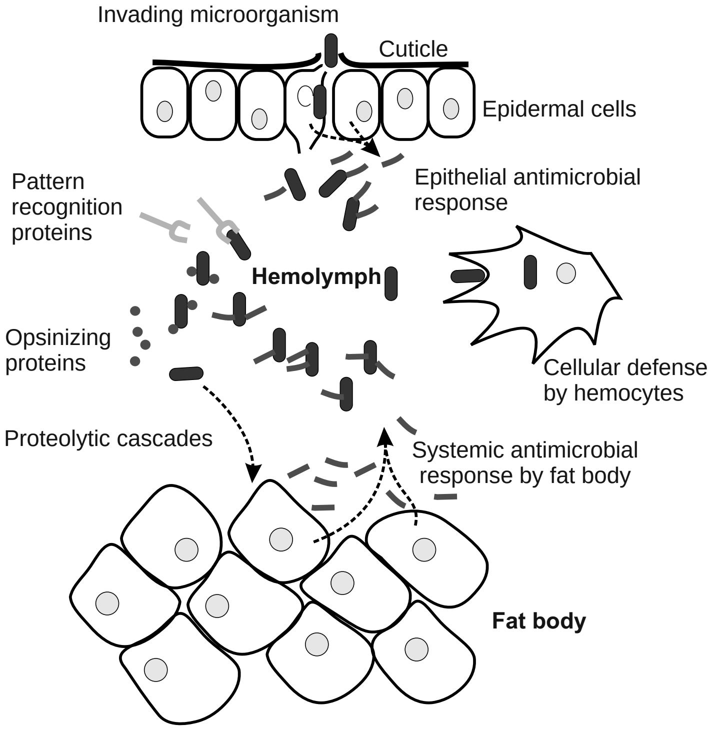

FIGURE 7.17. Possible mechanisms of response to invading parasites by epidermal cells, hemocytes, and fat body. Epidermal cells produce antimicrobial compounds, and pattern recognition and opsinizing proteins target invaders for attack by hemocytes. Fat body cells can also mount a systemic antimicrobial response.

mechanism to a much greater degree than vertebrates, whose closed circulatory systems can easily be obstructed by blood clots. Enzyme cascades in the blood of arthropods produce clots by cross-linking proteins, first as a soft clot that later hardens with the action of hemolymph phenoloxidases. **Phenoloxidase** catalyzes the conversion of phenols to quinones, but it is first present as an inactive **prophenoloxidase** precursor that must be proteolytically activated by the terminal component of the cascade, a serine protease, called **prophenoloxidase-activating enzyme**, which is in turn activated by conserved components of the microorganisms that are recognized. The proteins in the surrounding cuticle thus become sclerotized, which can more effectively block the invasion of microorganisms (Figure 7.18).

Lectins are multimeric carbohydrate-binding proteins consisting of 30 to 40 kDa subunits that are capable of agglutinating vertebrate red blood cells. Their production in insects is induced by injury, and they circulate within the plasma of many insects and bind to the carbohydrates in the cell walls of

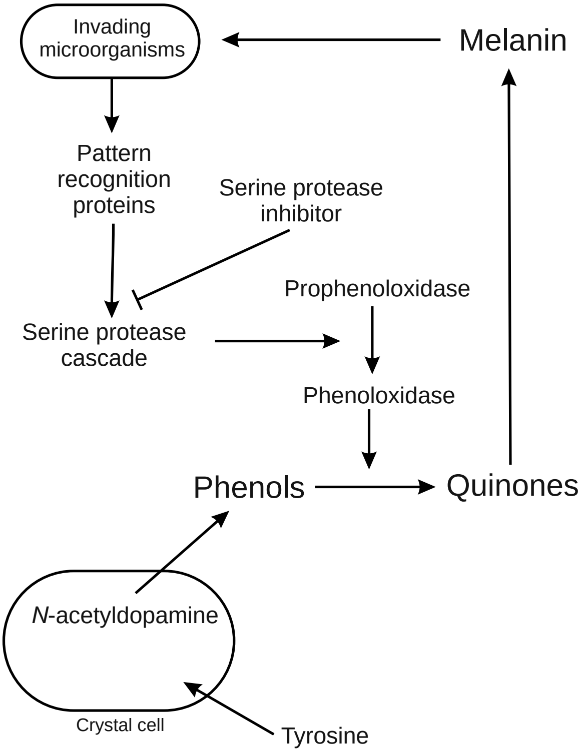

FIGURE 7.18. Mechanism of humoral encapsulation by the deposition of melanin on foreign invaders. The serine protease inhibitor restricts phenoloxidase activity to the site of the infection.

microorganisms. With their multiple binding sites, they can cause an aggregation of lectin-linked cells. The binding of the lectins to the surface of bacteria may be an initial signal of **opsinization** in which they are marked for recognition and mobilization by hemocytes.

Many of the immune pathways are initiated by pattern recognition molecules that allow the insect to distinguish self from nonself. Specific pattern recognition receptors that respond to components in microorganisms such as peptidoglycans and lipopolysaccharides have been identified. A **peptidoglycan recognition protein** (PGRP) that initiates the cascade leading to melanization was first isolated from the hemolymph of *Bombyx mori* larvae and has since also been identified in *Drosophila* and humans. Gram-positive microorganisms are detected by a circulating PGRP, whereas a transmembrane PGRP appears to bind gram-negative organisms. *Drosophila* has at least 13 different PGRPs that are similar to those in *Bombyx, Trichoplusia*, humans, and rats. These PGRPs are critical components in the signaling pathways that are activated by infection.

Six homologues of **thioester-containing proteins** (TEPs) have been identified in *Drosophila* and 19 from the mosquito *Anopheles gambiae*. In the immune systems of vertebrates, they play important roles as components of the complement system, consisting of about 30 proteins that act on invading microorganisms. A cascade of activation steps occurs at the bacterial surface, ultimately inserting channels in the bacterial membrane that cause it to lose its integrity. In insects, TEPs opsinize gram-negative bacteria for phagocytosis, displaying a complement-like function.

The adaptive immune system of higher vertebrates is capable of producing a huge number of diverse antibodies through the recombination of somatic genes, a capability previously not believed to exist in invertebrates. Insects display a very basic form of **innate immunity**, consisting of the ability to distinguish between self and nonself, which is a critical first step in immunological defense. Innate immunity is an evolutionarily ancient form, depending on genetically encoded factors that recognize conserved features of microorganisms and activate immediate cellular and humoral defense responses. Insects do not produce the immunoglobulins that mediate **adaptive** or **acquired immunity**, which involves somatic gene rearrangements that produce unique clonal antigen receptors following an infection. Genes encoding the molecules of acquired immunity have so far only been identified in sharks and higher vertebrates. Innate immunity is far more efficient than adaptive immunity, requiring fewer genes than for immunoglobulins and having a faster reaction time. The innate immunity of insects was once thought to be nonspecific, but it is indeed capable of producing an array of fairly specific humoral substances that combat infection.

Insects have immunoglobulin-like molecules that show a high degree of immune receptor diversity and the ability to generate a large number of recognition elements. The ***Dscam*** gene of *Drosophila* is a homolog of the vertebrate

Down syndrome adhesion molecule, an immunoglobulin superfamily member that serves as an adhesion molecule in nervous tissue, is involved in axon migration and neural differentiation, and whose name is derived from its responsibility for the peripheral nervous system defects of Down syndrome. The *Drosophila* homolog of the *Dscam* gene, as a consequence of its alternative splicing, has the potential for generating multiple mRNAs from the single gene and can thus encode a diverse number of isoforms. With 116 coding regions, the gene can potentially encode for more than 38,000 of these different protein isoforms that specify neural identity and participate in the specificity of neuronal connectivity. In addition, these *Dscam* isoforms have also been identified in the hemocytes and fat body cells of *Drosophila*. Different isoforms bind bacterial cells with different affinities, and their experimental loss from hemocytes impairs the efficiency of these cells to phagocytose bacteria. The possibility that these immunoglobulin-like molecules may function as primitive antibodies in insects is intriguing and suggests they might be an early step in the evolution of immunity.

To limit the growth of invading pathogenic bacteria, many mammals withhold iron, a necessary component for their growth. They employ a group of iron-binding proteins in the blood, milk, and tears known as **transferrins** to bind and sequester the iron. Insects also sequester iron in extracellular fluids when infected with bacteria, and although the evidence for its role is less clear than in mammals, insect transferrins that are sequestered in the hemolymph may play a role as antibiotic agents.

Cell-Mediated Immunity

The role of hemocytes in the resistance of insects to invading microorganisms has been known since research by Metchnikow in the late 1800s. Hemocytes are able to recognize some foreign bodies in the hemolymph and phagocytose them. Plasmatocytes and granulocytes are the major cell types involved in this phagocytosis. The process first involves the recognition of foreignness, based on surface receptors on the hemocyte membrane. This recognition is followed by the formation of pseudopodia and the ingestion of the foreign particles within a membrane-bound phagosome. After the phagosome moves to the cell interior, it fuses with a lysosome where the foreign tissue is digested and destroyed by the agents that are released.

The response of hemocytes to larger foreign bodies that cannot be phagocytosed, either those living or nonliving, is to isolate them from the other insect cells by the process of **encapsulation**. Through encapsulation, multiple layers of hemocytes wall off the foreign object and prevent it from contacting host cells and sources of oxygen and nourishment. The plasmatocytes and granulocytes are also involved in this defensive response that begins with the recognition

of foreignness of an invading body of cells. Once recognized, the invader triggers the release of chemotactic aggregation factors from granulocytes that bring more plasmatocytes to the site. Initially surrounding the foreign body, the plasmatocytes flatten out and die. As more plasmatocytes attach to the site, they also flatten but remain alive, walling off the body with a layer of cells as deep as 50 or more. The recruitment of hemocytes ends when the capsule that is formed becomes coated with glycoaminoglycans similar to that of the basement membrane that covers all tissue surfaces in the hemocele. Plasmatocytes contain the enzyme phenoloxidase and deposit the melanin they synthesize, further walling off the invader. Larval *Drosophila* parasitized by a wasp egg envelope the egg with lamellocytes and neutralize it by depositing melanin on its surface. Larvae are not immunocompetent until the third instar, when 20-hydroxyecdysone triggers the proliferation, differentiation, and dispersal of hemocytes from the lymph gland.

A **humoral encapsulation** is also possible in which melanin is deposited on the foreign surface in the absence of participation by hemocytes. In *Drosophila,* crystal cells release an inactive prophenoloxidase into the hemolymph, which is activated to phenoloxidase by a serine protease cascade that then catalyzes the conversion of phenols to quinones (Figure 7.18). The intermediary quinones are toxic to invaders and also polymerize to produce melanin. The phenoloxidase activity must be restricted to the site of infection so that the rest of the insect's cells are not exposed to these toxins; therefore, in the absence of infection, serine protease inhibitors, or **serpins**, inhibit the serine proteases and prevent the prophenoloxidase from becoming activated. After microorganisms invade the hemocele, the serpins localize its activity by remaining active in noninvasion areas to prevent the excessive activation of the prophenoloxidase that might otherwise damage insect host cells. An example is the protein *Serpin27A*, which inhibits the enzyme cascade that activates prophenoloxidase; *Spn27A* mutants show melanization in the absence of invasion and completely melanize after injury. Serpins are also involved in the developmental pathway that establishes dorsal-ventral polarity during embryogenesis. Humoral encapsulation has only been reported to occur in a few insects that have relatively small populations of hemocytes and is a fast and efficient way to deal with invading bacteria and fungi.

The lymph glands of larval *Drosophila* release large numbers of pupal macrophages at metamorphosis that are instrumental in the remodeling process. They phagocytose cells of larval structures that are programmed to degenerate during metamorphosis, including the larval lymph gland itself. The differentiation of these macrophages is dependent on 20-hydroxyecdysone release.

Nodule formation may also trap large numbers of invading bacteria. Nodules consist of aggregations of hemocytes that produce extracellular material that form a matrix to catch large numbers of bacteria that are too big to be phagocytized. Larger nodules may eventually also be encapsulated.

Nephrocytes are mesodermal cells found throughout the hemocele that are able to sequester high-molecular-weight colloids but not bacteria. Included in this category of cells are the **pericardial cells**, the best known of the nephrocytes. They are primarily located on each side of the heart, attached to the dorsal vessel and alary muscles by connective threads, but they are sometimes numerous around the fat body as well. These cells absorb chemicals by pinocytosis and return the degraded substances to the hemolymph. They contain numerous granular inclusions. In addition to this detoxification function, there is evidence that the pericardial cells also synthesize and secrete hemolymph proteins.

Humoral Immunity

To supplement the cell-mediated immunity from hemocytes, insects produce a battery of antimicrobial peptides that are synthesized by the fat body and are released into the hemolymph at the time of a microbial infection. It has long been observed that the initial injection of heat-killed pathogenic bacteria results in a reduced insect mortality when live bacteria are subsequently injected. The injection of peptidoglycans or lipopolysaccharide from bacterial cell walls into *Manduca* or *Bombyx* larvae induce the same synthesis of various antibacterial hemolymph proteins by the fat body as do whole bacteria. The response is fairly specific; whereas a fungal infection might result in the induction of the antifungal peptide drosomycin, it fails to induce the antibacterial peptide diptericin. The immune system of the bumblebee is primed after an initial exposure to bacteria, and when reinfected several weeks later after the initial infection has been cleared, the system responds with a narrow specificity and increased protection against that specific bacterial species. Antimicrobial peptides appear as soon as 2 to 4 h after a septic injury and can be synthesized at a rate that is about three times faster than the reproductive rate of bacteria, allowing the insect to eliminate the invaders. Individual insects may produce 10 to 15 different antimicrobial peptides, with each displaying a different activity spectrum (Figure 7.19).

Antimicrobial peptide famiy	Acts against
Attacin	Antibacterial (Gram -)
Cecropin	Antibacterial (Gram -)
Defensin	Antibacterial (Gram +)
Diptericin	Antibacterial (Gram -)
Drosomycin	Antifungal
Drosocin	Antibacterial (Gram -)
Metchnikowin	Antifungal

FIGURE 7.19. General activity spectra of antimicrobial peptide families.

Antimicrobial peptide genes expressed in fat body cells are upregulated by immune challenge and are under the control of two nuclear factor signaling pathways, Toll and immune deficiency. The activated nuclear transcription factors that modulate gene expression in both pathways are members of the **NF-κB** family of transcription factors that are also functional in mammals. The **Toll signaling pathway** regulates the expression of genes that encode antifungal and antibacterial peptides. Toll is believed to be the ancestral immune signaling pathway in insects and regulates several components of immunity. Gram-negative bacteria activate the immune response of insects through the **immune deficiency** (IMD) pathway that regulates the expression of genes that encode additional antibacterial peptides. The evolution of two distinct pathways in fat body cells appears to be an efficient mechanism to tailor the immune response of insects to different invaders.

The Toll receptor involved in the *Drosophila* immune response is a transmembrane receptor that was identified first as a component in the developmental pathway that establishes the dorsoventral axis during *Drosophila* embryonic development. The name "Toll" comes from the first gene that was identified in the pathway. So far, nine different Toll receptors have been identified in *Drosophila.* The Toll protein has an intracellular signaling domain, TIR, that is similar to both the human interleukin-1 receptor and plant disease resistance genes, but unlike in mammals where it functions exclusively in immunity; insects also employ the Toll pathway during embryonic development. The mammalian Toll pathway is initiated by a 10-member family of **Toll-like receptors** (TLRs) that interact directly with microbial patterns. In contrast, insect Toll is activated by its ligand, Spätzle, a circulating protein that is first activated by a serine protease cascade following an infection. Interestingly, Spätzle is a homolog of growth factors and peptides that are also associated with plant defense. A serine protease inhibitor, or **serpin**, prevents the activation of the Toll pathway in the absence of immune challenge. Once bound by Spätzle, Toll activates a cytoplasmic cascade that ultimately results in the translocation of the transcription factors to the nucleus and the subsequent transcription of antimicrobial gene products such as drosomycin that are active against gram-positive and fungal infections (Figure 7.20).

The IMD signaling pathway culminates in the transcription of antimicrobial gene products that are active against gram-negative microorganisms. The gram-negative bacteria are recognized by the transmembrane peptidoglycan receptor PGRP-LC, with the possible involvement of additional signaling from hemocytes. The pathway culminates in the activation of a transcription factor that enters the nucleus and activates gene transcription of the antibacterial peptides diptericin and cecropin A (Figure 7.20).

The first of the antibacterial peptides to be characterized were the **cecropins**, isolated in 1980 from the pupae of *Hyalophora cecropia.* These peptides have since also been isolated from mosquitoes and *Drosophila.* The cecropins are low-

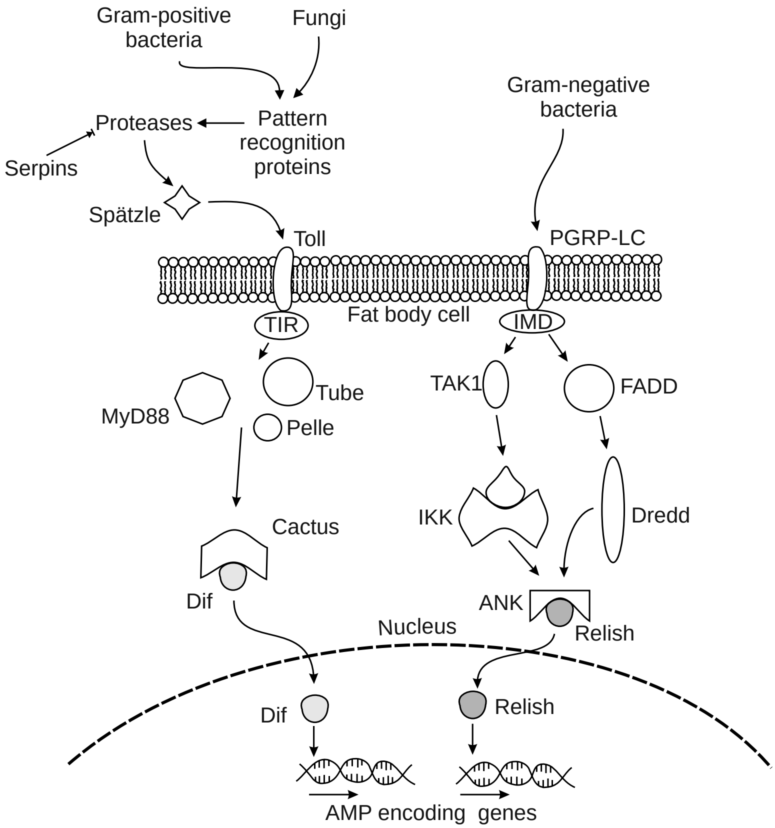

FIGURE 7.20. The Toll (*left*) and IMD (*right*) signaling pathways. In the Toll pathway, an infection activates proteases that in turn activate Spätzle. Serpins inhibit the proteases in the absence of an immune challenge. When Spätzle binds to the Toll receptor, an intracellular signaling cascade causes the degradation of Cactus and the translocation of the Dif protein to the nucleus, where it initiates the transcription of antimicrobial peptides (AMP). In the IMD pathway, bacteria bind to a transmembrane peptidoglycan recognition protein (PGRP-LC) that activates the IMD protein and initiates a signal transduction that ultimately leads to the translocation of the Relish transcription factor that regulates AMP genes.

molecular-weight peptides (<5 kDa with between 35 to 39 amino acids), most of which are basic at the N-terminal and give the molecule a net positive charge. They are active against both gram-positive and gram-negative bacteria as membrane-active antibiotics that create channels in their lipid bilayer. Cecropins may also be active in insect vectors against the parasites that cause malaria and might possibly contribute to the refractoriness to infection by some mosquitoes.

The **defensins** are small 29- to 34-residue peptides of between 4–5 kDa, homologs of which have been identified in mollusks and scorpions and are

widely distributed in mammals as well as in insects. There are more than 40 varieties of insect defensins, also sometimes referred to as **sapecins**, which are active against gram-positive bacteria. As with cecropins, the insect defensins disrupt the permeability of the bacterial membrane, killing them instantaneously. Some mosquitoes also produce defensins that can interfere with the potential development of the protozoan malaria parasite.

The *Drosophila* fat body synthesizes the defensin-like **drosomysin** within 30 min of infection. Drosomysin consists of 44 amino acids with eight cysteine residues associated with four disulfide bridges. It has more of a structural similarity to plant defensins than to insect defensins and is similarly active against filamentous fungi but is ineffective against bacteria and yeasts.

Other inducible antibacterial peptides belong to proline-rich or glycine-rich families. The proline-rich peptides are active mostly against gram-negative bacteria and are relatively small, containing 15 to 34 residues and a high proportion of proline and arginine. They include the **apidaecins** from the honey bee, **formaecins** from the ant, **drosocins** and **metchnikowins** from *Drosophila*, **pyrrhocoricins** from the sap-sucking bug, and hemipteran **metalnikowins**. Rather than affecting bacterial membrane permeability and killing immediately, these peptides may bind to bacterial proteins and take longer (6 to 12 h) to kill.

The glycine-rich family of peptides has larger members, ranging from 8–30 kDa, and contain a high proportion of glycine residues. These are active primarily against gram-negative bacteria, perhaps by inhibiting the synthesis of outer membrane proteins that results in increased membrane permeability. Included in this family are the various **attacins**, **sarcotoxins**, **coleoptericins**, **hemiptericins**, **diptericins**, **gloverins**, and **hymenoptaecins**, named after the insect from which they were isolated.

Hyalophora cecropia and *Manduca sexta* pupae produce **hemolin**, a 47-kDa carbohydrate-binding protein, in response to infection. Hemolin is among the first proteins to bind to the surface of invading bacteria and forms a protein complex that may initiate the immune response. Its amino acid sequence contains four internal repeats that are characteristic of immunogloblin-like domains and place it in the immunoglobulin superfamily. Hemolin inhibits the aggregation of hemocytes, suggesting that it may affect their adhesive properties during a defensive response and convert them to an activated state. This may be a mechanism for opsonization or for trapping bacteria in nodules.

By its synthesis of a battery of antimicrobial substances that are similar to those produced by vertebrates and plants, the fat body is able to respond quickly to infections by microorganisms. These substances have even been proposed as candidates for dealing with the problem of drug resistance in human bacterial infections. They rapidly kill a broad array of pathogens, do not affect mammalian cells, and have a mode of action that minimizes the development of resistance.

THE CIRCULATORY SYSTEM AND TEMPERATURE VARIATIONS

As small terrestrial animals that thrive in temperate climates, insects have also successfully adapted to the cold temperatures of winter. Some insects have evolved the ability to migrate to warmer climates when they recognize the signals of seasonal photoperiod. Others remain in the cold, having evolved physiological adaptations to subzero temperatures. Although low temperatures can affect the physical properties of biologically important molecules and the rate of biological processes, the exposure to freezing temperatures itself is not necessarily detrimental. However, when the low temperatures induce the formation of intracellular ice crystals, physical damage that cannot be repaired can occur to cell membranes and is generally lethal for the organism. In addition to postural adjustments to better absorb solar radiation on cool days, insects use the heat generated by flight muscles and distributed by the circulatory system to raise body temperatures.

Cold Hardiness

Cold hardiness describes the ability of insects to survive exposure to low temperatures. Insects can tolerate winter temperatures if they first undergo a physiological preparedness that may take several weeks to develop. The acclimatization process occurs when insects are first exposed to low temperatures, which trigger the accumulation of the cryoprotectants. Their production typically occurs in the early autumn, remains at a plateau during the winter, and declines by early spring. A short-term exposure to cold temperatures can also protect some insects from subsequent lethal temperatures. This **rapid cold-hardening** response significantly increases the chance of survival of an insect at a low temperature if it is preexposed to a less severe low temperature. Insects that live in variable environments may use this rapid cold-hardening response to survive sudden cold temperatures. A sarcophagid fly larva can withstand temperatures as low as −10°C if first chilled for 30 min at 0°C.

Two general physiological strategies have been recognized in species that successfully overwinter. Insects that are categorized as being **freeze tolerant** are able to withstand the formation of extracellular ice crystals. They synthesize ice-nucleating proteins that raise the supercooling point of body fluids and serve as catalysts for the nucleation of ice in safe extracellular areas. Water moves from the cells to these extracellular areas, preventing intracellular freezing from occurring. The extracellular freezing that occurs at the raised temperatures (generally above −8°C) gives the cells time to adjust to the osmotic changes that result from the formation of ice crystals and reduces the likelihood of intracellular freezing. Freeze-tolerant insects also synthesize carbohydrate cryoprotectants,

often in pairs of glycerol and sorbitol. Trehalose is also a common cryoprotectant that stabilizes cell membranes. The biosynthesis of all known carbohydrate cryoprotectants begins with glycogen (Figure 7.21).

Freeze-avoiding species produce hemolymph cryoprotectants that allow the insect to supercool and remain in a liquid state without the formation of ice crystals. These species can often supercool to as low as −35°C. Gall-forming larval dipterans in northern Canada are capable of supercooling to as low as −60°C. The cryoprotectants that are produced include glycerol, sorbitol, trehalose, and mannitol often in concentrations approaching 25% of the insect's total body weight. In addition to preventing the formation of ice, these components may stabilize enzymes and cell membranes. There appear to be more species that have gone the route of freeze tolerance, perhaps because freeze avoidance may be energetically more costly.

A number of freeze-avoiding insects, including moths and beetles, produce **antifreeze proteins** (AFPs) that can lower the freezing point of water, yet have no affect on its melting point. AFPs in the hemolymph and gut inhibit the ice nucleators that seed ice crystal formation and also help stabilize cell membranes. These proteins are high in threonine and cysteine and are able to bind to ice crystals and prevent them from growing. AFPs were first identified from the blood of Antarctic fish and are common in Alaskan insects and spiders.

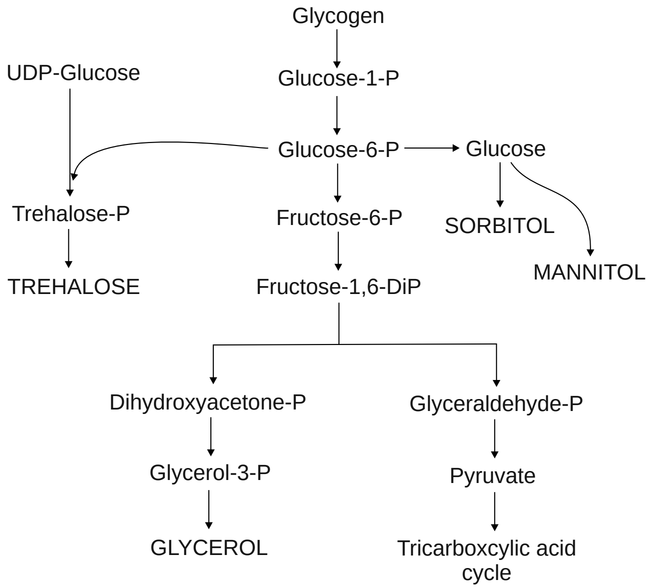

FIGURE 7.21. Biochemical pathways for the synthesis of hemolymph cryoprotectants. From Storey (1988).

Thermoregulation

Insect activity is largely restricted to the warmer parts of the year, but a few insects, such as gryloblattids and antarctic collembolans, are active during the winter months because their enzyme systems have been tuned to optimal activity at those lower temperatures. Most other species are completely at the mercy of environmental temperatures and are inactive when the temperatures are low. However, some insects are able to regulate their internal temperatures by capturing metabolic heat and using the circulatory system to distribute that heat, so they may be active when ambient temperatures are too low to otherwise allow this activity to occur.

Temperature maintenance may be based on either external or internal sources. **Ectothermy**, based on external heat acquisition, is the more primitive means of thermoregulation in nonflying insects, occurring when the insects position themselves in an area of preferred temperature. By basking, behaviorally regulating their exposure to the sun, they actively control the temperature of their bodies. Increased body pigmentation may contribute to the absorption of solar radiation. In contrast, **endothermic** insects use the heat generated from flight muscles to raise their body temperatures. Although insect flight muscles are the most metabolically active tissue known, they have a mechanical efficiency of no more than 20%, and the remaining 80% of energy expended during flight is degraded as heat that can be used to regulate body temperature. Almost inconsequential in smaller insects, the generation of heat by the flight muscles of larger insects can be substantially higher than ambient levels.

The flight muscles of larger insects must operate at high enough temperatures to produce the sufficient lift for flight to occur, but the reliance on the flight muscles to generate heat to raise body temperature presents a bit of a paradox. Insects can use flight muscles as a source of heat, but no flight is possible at low ambient temperatures to provide this heat. The problem has been solved by a **preflight warmup** in which antagonistic flight muscles contract simultaneously to generate the initial heat that allows them to reach their temperature optima. The behavior is sometimes known as **shivering** because the contractions may cause the wings to quiver slightly. Bumblebees require a thoracic temperature above 30°C before the muscles can generate sufficient lift; by shivering, they can raise their temperatures to this point even when ambient temperatures are as low as 3°C. Social insects are able to overwinter by trapping the metabolic heat produced by clustering individuals. On a cool day, honey bees at the core of a cluster maintain temperatures of 30° to 40°C, whereas those at the periphery remain at 15°C. Before the insects swarm to a new home, this temperature gradient disappears, and all bees attain the thoracic temperature of 33° to 35°C necessary for flight.

The heat is largely concentrated in the thorax, maintaining the flight muscles at the higher temperatures even though the circulatory system is pumping cooler

thoracic hemolymph into the warmer thorax. The temperature differential is maintained by the operation of a **countercurrent heat exchange** in the circulatory system. In the bumblebee, a narrow petiole connects the thorax and the abdomen, and through it pass the dorsal vessel, the digestive tract, the tracheae, and the ventral diaphragm, all closely appressed (Figure 7.22). Blood is pumped forward within the dorsal vessel from the cool abdomen through the warm flight muscles in the thorax and into the head. The warm blood then moves posteriorly within the hemocele through the petiole and back into the abdomen, with the ventral diaphragm regulating its rearward passage. To maintain the heat in the thorax, contractions of the ventral diaphragm and dorsal vessel are timed so that streams of hemolymph both moving forward and backward pass through the petiole simultaneously. As they pass each other, some of

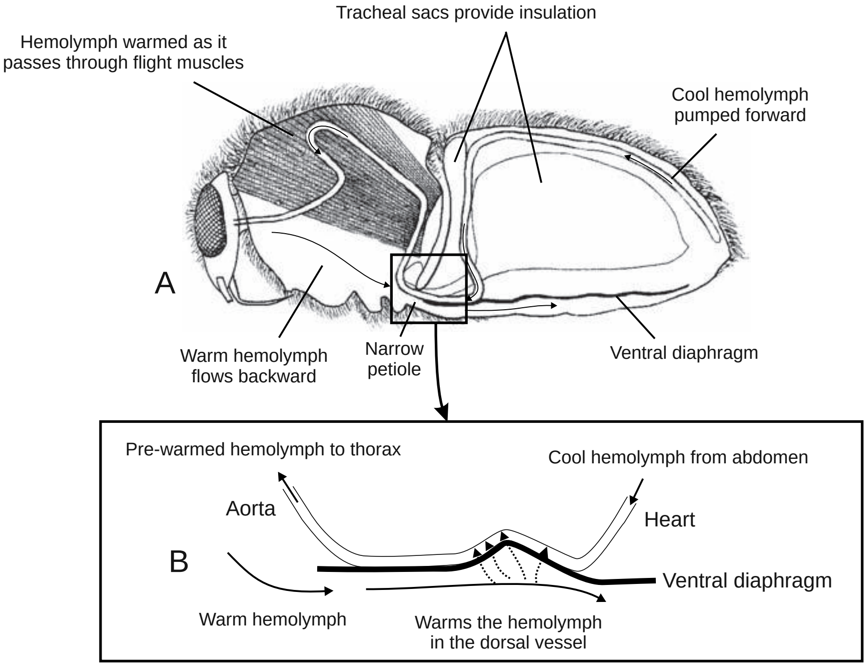

FIGURE 7.22. A. Longitudinal section of the bumblebee, showing the path of the dorsal vessel through the narrow petiole and the flight muscles. B. The countercurrent heat exchange occurs with the passage of cool hemolymph from the abdomen within the heart at the same time that warm hemolymph flows backward in the hemocele. The warm hemolymph from the thorax prewarms the hemolymph from the abdomen before its heat can be dissipated when it flows into the abdomen. From Heinrich (1976). Reprinted with permission.

the heat acquired by blood in the thorax is passed to the cooler blood from the abdomen, warming it before it enters the thorax. The countercurrent exchange thus keeps most of the heat in the thorax. The winter moth, *Eupsilia morrisoni,* can be airborne on days when the temperatures are as low as 0°C because it can maintain its thorax at temperatures between 30° and 35°C. These moths are especially efficient in maintaining elevated thorax temperatures because in addition to the heat exchanger in the anterior abdomen, a countercurrent heat exchanger is present in the thorax (Figure 7.23).

On warm days when there is no need to maintain a warmer thorax, the countercurrent exchange in bumblebees is reduced so that hemolymph passing forward through the petiole alternates with hemolymph passing backward (Figure 7.24). This brings the warmer hemolymph into the abdomen where it can be dissipated. The bee can also use this heated abdomen to incubate the brood. At higher ambient temperatures, honey bees are able to reduce their metabolic heat production by regulating their wing-beat frequency and reducing it when necessary so as to lessen the accumulation of internal heat.

Japanese honey bees use the heat they generate for defense. The giant hornet, *Vespa mandarinia japonica,* preys on many social bees and wasps, decimating the colonies and capturing the brood to feed to their own larvae. The Japanese honey bee, *Apis cerana japonica*, has evolved a defense in which 500 or more workers engulf a single hornet invader in a ball. The internal temperature of the ball is raised to 47°C, which is lethal for the hornet but not for the bee.

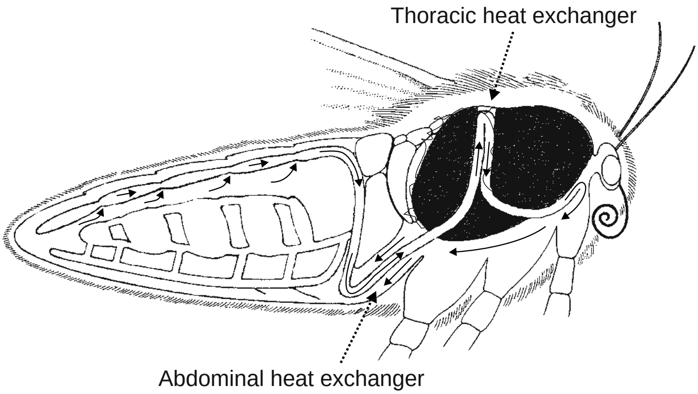

FIGURE 7.23. A second heat exchanger in the thorax of winter moths. From Heinrich (1987). Reprinted with permission.

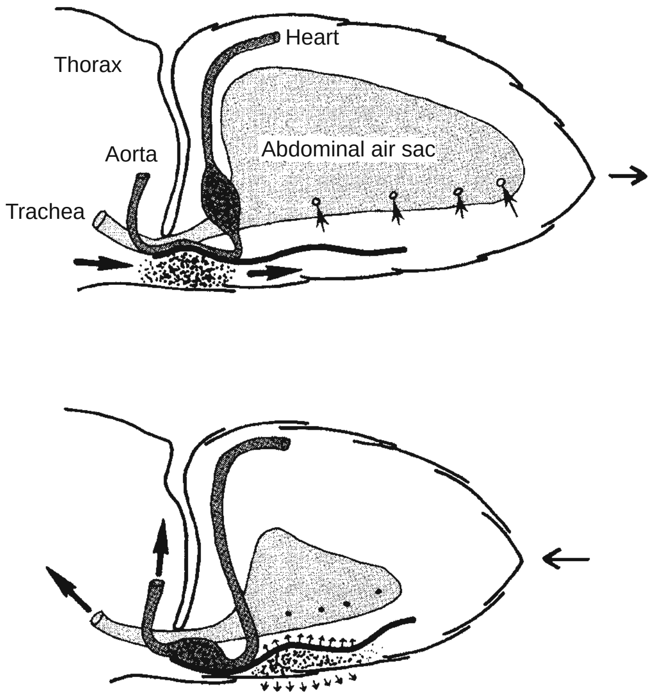

FIGURE 7.24. The countercurrent heat exchange is reduced in bumblebees when the insect must rid itself of heat on a warm day. The passage of warm hemolymph backward from the thorax alternates with cool hemolymph passing forward to the thorax. Hemolymph movements are coordinated by the inflation of abdominal air sacs and the extension and contraction of the abdomen. From Heinrich (1976). Reprinted with permission.

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Excretory Systems

The excretory system maintains the internal environment of the insect by separating and eliminating metabolic wastes and other toxic substances from body compartments. Because these wastes are often dissolved in water, excretory processes are also closely associated with osmoregulation and the maintenance of water balance. Insects are typically 65% to 75% water by wet weight but can range from 17% to 90% during the various life stages of particular species. An example of the degree to which insects can withstand dehydration is a chironomid midge that can survive dehydration down to a moisture content of 3% then withstand heating to 104°C and resume its life processes when rehydrated. Those dehydrated to 8% are able to survive cooling to −270°C in liquid helium and grow normally when immersed in water. What other animal is capable of surviving such water deficits and temperature extremes?

Insects are able to occupy a wide variety of terrestrial niches and effectively deal with issues of water balance largely because of two important adaptations: their impermeable exoskeleton and an excretory system of considerable sophistication. As small terrestrial animals with a high ratio of surface area to body volume, insects lose water readily and this loss must not be compounded by efforts to get rid of wastes. On the positive side of the ledger, their small size allows them to easily escape to more favorable microclimates.

MAJOR EXCRETORY PRODUCTS IN INSECTS

Insects ingest a wide variety of foods from which they derive energy. When these foods are broken down and the nutrients assimilated, there are usually products left over that must be eliminated or sequestered to prevent them from accumulating and eventually poisoning other metabolic pathways. When carbohydrates or lipids are ingested, the energy captured from their oxidation is accompanied by the end products of carbon dioxide and water, neither of which causes any great problems with accumulation or toxicity. Carbon dioxide easily diffuses through soft insect cuticles or is eliminated through the tracheal system. Excess water is generally not a problem and can be easily eliminated when it accumulates.

In contrast, the metabolism of proteins and nucleic acids yields nitrogen in addition to carbon dioxide and water. Amino acids cannot be stored to as great a degree as carbohydrates and lipids, and when more protein is consumed than is needed for maintenance, the excess nitrogen must be eliminated quickly. The nitrogen itself is not toxic, but in biological systems it readily forms ammonia. Some of this ammonia can be recycled into amino acid synthesis by the formation of glutamate from α-ketoglutarate and glutamine from glutamate (Figure 8.1), but the excess ammonia that remains is highly toxic unless it is diluted with water. High levels of ammonia can interrupt nervous transmission by substituting for necessary potassium and can also alter carbohydrate and lipid metabolism. Ammonia dissolves easily in water to form ammonium hydroxide, which is caustic and disrupts cell membrane lipids.

Animals must therefore have excretory systems that are designed to prevent the toxic accumulation of ammonia. Because ammonia is very soluble in water, it is difficult to sequester from critical biological reactions. It crosses biological membranes easily, and concentrations must be maintained below toxic levels by dilution with water. Generally, for each gram of ammonia that is retained within an organism, about 400 ml of water is necessary to reduce it to this safe level. Aquatic insects have little trouble finding these large quantities of water for dilution, and many even excrete the nitrogen from protein metabolism directly as ammonia. However, terrestrial organisms are unable to carry a water supply that is large enough to dilute the ammonia, and there are needs for both water conservation and the incorporation of the nitrogen into a less toxic molecule than ammonia. Most terrestrial organisms have adopted the pathways of incorporating nitrogen into either **urea** (**ureotelic** animals) or **uric acid** (**uricotelic** animals) (Figure 8.2). Many mammals possess the enzyme uricase that breaks down uric acid into **allantoin**, which is much more soluble and easily excreted by the kidneys. In primates, birds, and reptiles, the uricase gene is not expressed, and uric acid remains as the end product of purine metabolism.

As a consequence of their lower toxicity, these molecules can be concentrated in body fluids to a much greater extent than can ammonia and require less water

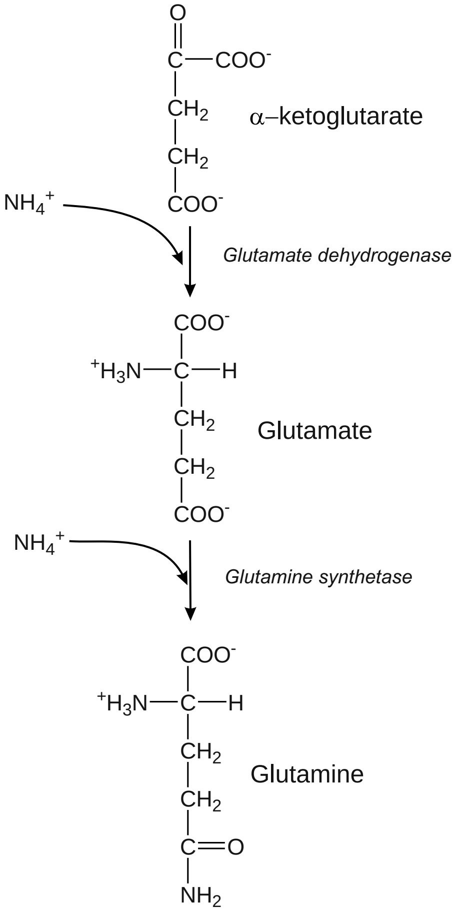

FIGURE 8.1. The incorporation of ammonia in the synthesis of amino acids.

for dilution. Urea is more soluble than ammonia but far less toxic and requires about 10 times less water to be diluted to nontoxic concentrations. For a relatively large terrestrial animal that has easy access to water or that can carry sufficiently large water reserves, urea is an adequate molecule into which the nitrogen may be incorporated. However, in insects, the need for water conservation may have been the driving force for the incorporation of their nitrogen wastes into uric acid, which is an ideal excretory product for small terrestrial animals. Uric acid is highly insoluble in water and therefore fails to reach toxic levels in body fluids, so it requires about 50 times less water to dilute than does ammonia. It is only slightly soluble in biological fluids below pH 7 but becomes much more soluble above pH 9.5, and its insolubility in water allows it to be

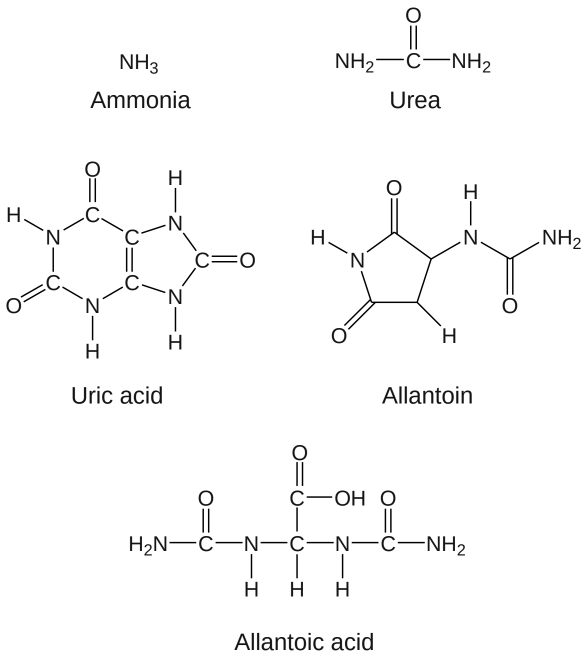

FIGURE 8.2. Excretory molecules that incorporate nitrogen.

excreted in a dry form without having a significant effect on water balance. Because hydrogen atoms are derived from water, their loss when incorporated in an excretory molecule affects water balance. Uric acid has the lowest ratio of H : N (1 : 1) for any of the excretory products, compared to the ratio of 2 : 1 for urea and 3 : 1 for ammonia.

Insects pay a high price for the benefits they derive from employing uric acid as a way to excrete nitrogen and still maintain a positive water balance. The synthesis of uric acid from protein results in the loss of several carbon atoms that could be used for other biosyntheses and requires a substantial amount of energy to build a larger, less toxic molecule. Eight ATP are required to first make the intermediary metabolite, **inosine monophosphate** (IMP), synthesized by the successive addition of amino groups from glycine, glutamine, and aspartate (Figure 8.3). The IMP is then converted to uric acid through hypoxanthine and xanthine, the entry points for the catabolism of purines. It has been estimated that when the tsetse fly, *Glossina*, which feeds exclusively on nitrogen-rich blood, ingests a 100-mg meal, it uses the energy from 47 mg of the blood as overhead to dispose of the excess nitrogen through uric acid synthesis and excretion. In contrast, if the insect could excrete the nitrogen as ammonia, only 15 mg of the meal would be needed as an overhead cost of processing. Other terrestrial organ-

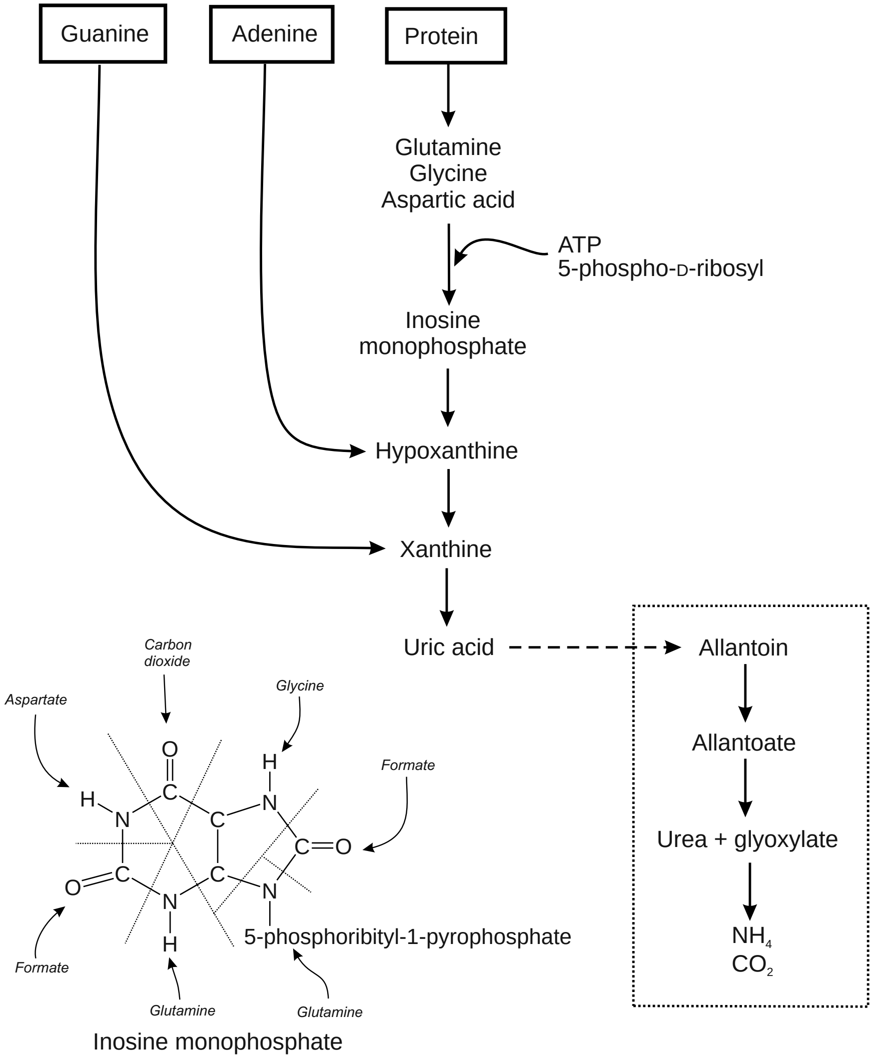

FIGURE 8.3. Pathway for the synthesis of uric acid from nucleic acids and protein. Inosine monophosphate requires ATP for its synthesis as well as several other building blocks incorporated into the molecule. The steps toward the synthesis of ammonia and carbon dioxide, shown in the box, do not take place in insects.

isms can convert uric acid to urea and finally ammonia and carbon dioxide, but insects lack the necessary enzymes.

Not all insects excrete uric acid, and not all excrete one product exclusively. The type of excretory product produced is often a function of diet, developmental stage, and ecological niche. Allantoin is excreted by the hemipteran

Dysdercus, and certain lepidopterans and larval dipterans excrete **allantoic acid** (Figure 8.2). Urea is also a minor component of some insects, but the complete pathway of urea synthesis in insects is not known. Although several enzymes of the ornithine cycle that operates in vertebrates have been identified, evidence for the complete cycle in insects is lacking. Arginine is converted to ornithine and urea by the enzyme arginase, which has been identified in insects, and the amount of urea excreted may correlate with levels of arginine in the diet. So when insects do excrete urea, it may result from the breakdown of dietary arginine (Figure 8.4). The ammonia that is excreted by some terrestrial insects, including blow fly larvae and some cockroaches and locusts, occurs as a result of the deamination of amino acids and not by the breakdown of urea (Figure 8.5). In the mosquito, *Aedes aegypti*, abundant nitrogen from blood is ingested each time it feeds, and a proline cycle is utilized to store the ammonia that is derived from amino acid deamination as proline and glutamine, which reduces the excretion of urea and uric acid (Figure 8.6). The proline can also be used to fuel flight. Gut bacteria may contribute to the varied excretory products of insects, but this is difficult to evaluate unless the insects are reared aseptically. Ammonia from a blood meal is used to convert glutamate to glutamine. Glutamine and α-ketoglutarate combine to yield two molecules of glutamate: one maintains the cycle and the other is involved in the synthesis of proline.

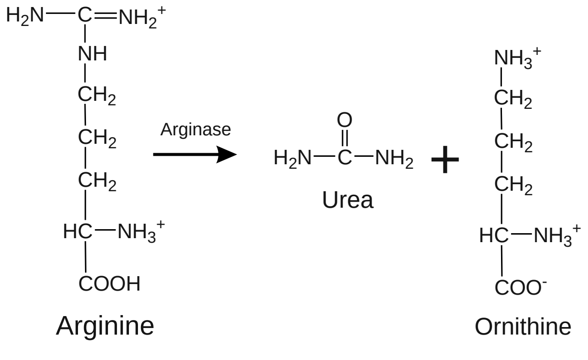

FIGURE 8.4. One way to account for the release of urea by some insects, with dietary arginine broken down to urea and ornithine. The complete pathway of the ornithine cycle in insects has yet to be identified.

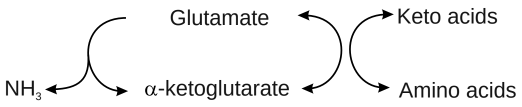

FIGURE 8.5. The release of ammonia by the deamination of amino acids.

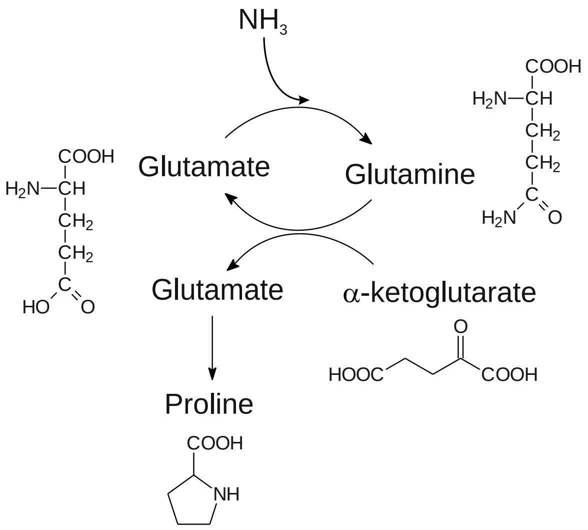

FIGURE 8.6. The incorporation of nitrogen derived from a mosquito blood meal. Ammonia from the blood meal is used to convert glutamate to glutamine. Glutamine and α-ketoglutarate combine to yield two molecules of glutamate, one of which maintains the cycle and the other is used to synthesize proline.

MALPIGHIAN TUBULES

As might be expected given the importance of osmoregulation in a small terrestrial animal, insects have evolved an excretory system of considerable sophistication. Analogous to the kidneys of vertebrates, the **Malpighian tubules** are the primary excretory organs of insects but operate in a different manner than do kidneys, which base their filtration on hydrostatic pressure. In the insect tubules, the driving force for excretion is the movement of ions across the epithelium. The Malpighian tubules are but one major part of the system that regulates salt and water balance in insects, with the other being the rectum. Excretion is a two-step process, with much of the fluid that is taken up by the tubules resorbed by the hindgut before it passes out of the body (Figure 8.7).

Their name is derived from Marcelo Malpighi, who first made reference to them as "vasa varicose" in 1669 in his work with the silkworm. Malpighi was the Pope's physician and also a comparative anatomist who used the newly invented microscope to also examine many other biological structures. Their function as excretory organs was not determined until 1815. Most of what we know about Malpighian tubule function is based on examinations of three insects: *Rhodnius prolixus*, *Aedes aegypti*, and *Drosophila melanogaster*.

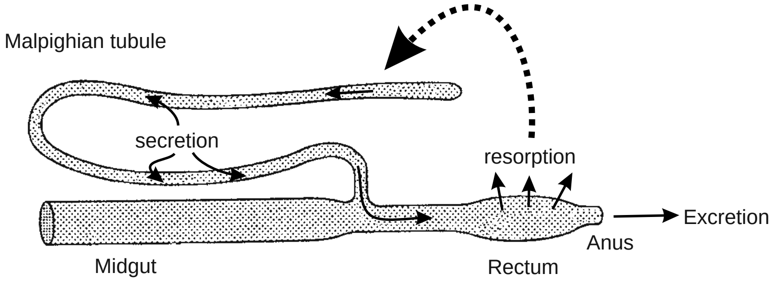

FIGURE 8.7. The overall mechanism of insect excretion. Fluid is taken up by the Malpighian tubules and moves to the hindgut, where the rectum resorbs some of the water, salts, and amino acids while the remainder is excreted.

The tubules are derived from two populations of cells that arise at the junction of the midgut and hindgut during embryogenesis: the ectodermal epithelial buds and mesodermal cells that are recruited as the structures develop. Thus, the insect Malpighian tubule is derived from two different cell populations, just as in the mammalian kidney. These two populations of cells, identified during development by their expression of transcription factors, give rise to the two major cell types of the tubules, the **principal cells** from ectoderm and the **stellate cells** from mesoderm (Figure 8.8) in a ratio of about 5 : 1. The principal cells have large intracellular concretions of metal complexes and long slender microvilli containing numerous mitochondria. They are active in the transport of sodium, potassium, and hydrogen ions as well as fluids. The stellate cells control the flow of chloride ions and lack both the concretions and mitochondria. Both show degrees of polyploidy, but there is considerably more endoreplication taking place in the principal cells. Both cell types may be further subdivided into distinct cell populations that can be found in different areas of the tubules.

The mature tubules may open directly into the midgut, anterior to the pyloric valve, or directly into the hindgut, as long tubes containing a hollow center, or lumen, that is closed at the distal end (Figure 8.8). Their walls consist of a single cell layer of polyploid cells and are differentiated by structure and function into several cell types along the length of the tubule. The four 2-mm long tubules of *Drosophila* are connected to the alimentary canal in pairs through the short **ureters** (Figure 8.8), and one of the connected pairs has an enlarged initial segment. In the blood-sucking bug, *Rhodnius prolixus*, the distal upper tubule contains binucleate cells with abundant microvilli and intracellular concretions that are aggregates of calcium, magnesium, and potassium, but cells of the lower tubule lack the crystals and have smaller microvilli. The upper tubules secrete ions and organic solutes into the lumen, and the lower tubule regions are resorptive (Figure 8.9). On the hemolymph side, the cells are covered by a basement

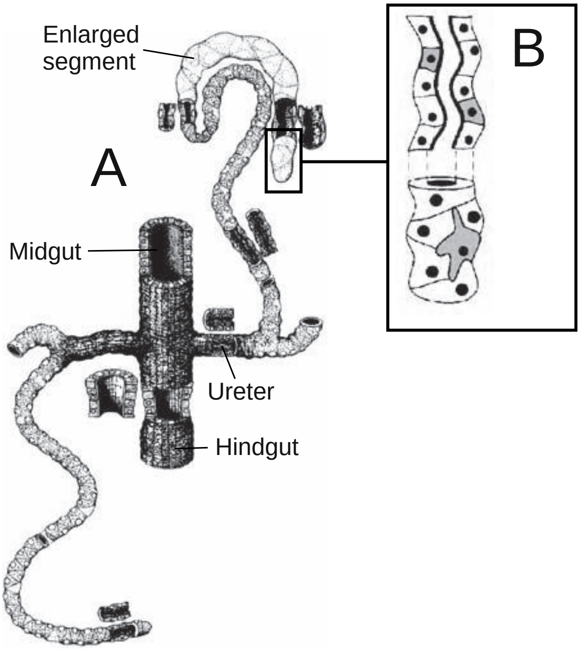

FIGURE 8.8. The Malpighian tubules of *Drosophila*. A. The four tubules are connected to the gut by the ureters. B. The tubules are made up of principal cells (unshaded) and stellate cells (shaded). From Wessing and Eichelberg (1978). Reprinted with permission.

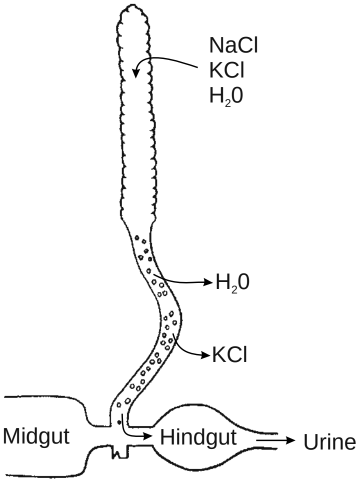

FIGURE 8.9. The mechanism of Malpighian tubule uptake and secretion. The distal regions of the tubules take up ions and water, and the proximal regions return water and selected ions back to the hemolymph. From Bradley (1998). Reprinted with permission.

membrane and a network of plasma membrane infoldings with trachea investing the tubules on the outside of the basement membrane. The cellular membrane that faces the tubule lumen contains a brush border with abundant microvilli that are rich in endoplasmic reticulum and mitochondria. These microvilli increase the surface area available for salt and water transport between the cell and the tubule lumen. The intermediate interior region of the cell contains numerous Golgi, rough endoplasmic reticulum, and vacuoles.

The tubules lie free in the body cavity and are surrounded by longitudinal muscles that allow them to engage in writhing movements that increase the contact between the tubules and the hemolymph. In some insects the distal ends of the tubule are physically associated with the hindgut, arranged into a **cryptonephridial system** that increases the resorption of water from the hindgut. This system will be described in more detail in a subsequent section. The total number of tubules varies in different species from 2 to more than 250, with a few species including aphids and collembolans that have none. There appears to be a direct relationship between the surface area of the tubules and the total body mass of the insect. The four tubules of *Drosophila* each have a length of approximately twice that of the whole body. In the cockroach, *Periplaneta*, there are 60 tubules with a total surface area of about 132,000 mm^2.

The Malpighian tubules initiate the excretory process. Their net uptake of fluid, ions, and waste materials is termed the **primary urine** and is transported into the tubule lumen (Figure 8.10). This primary urine travels through the lumen from the distal to proximal regions of the tubule and then to the alimentary canal where it mixes with the end products of digestion from the midgut. Generally isosmotic with the hemolymph, in most insects it contains a high concentration of K^+ and lower concentrations of Na^+ than are present in the hemolymph. In addition to excretory products such as uric acid, the primary urine may contain other ions, sugars, and amino acids that are resorbed later in

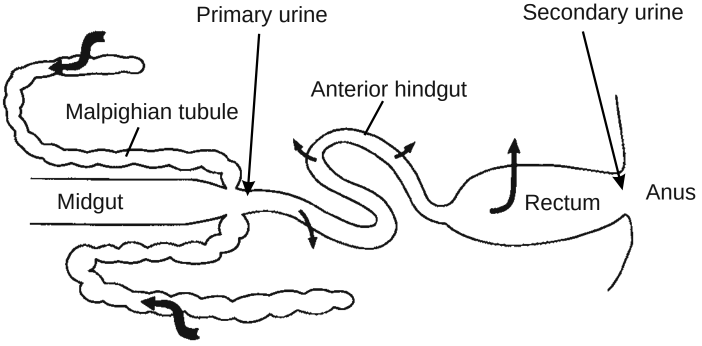

FIGURE 8.10. The formation of primary and secondary urine in the excretory system. From Maddrell (1981). Reprinted with permission.

the excretory process. In *Drosophila* larvae, the main segment is the only region reported to secrete K^+ fluid, with the lower tubule region resorbing water and K^+ and actively transporting Ca^{2+} into the lumen. The rapid secretion by the main segment in conjunction with the resorption that occurs in the lower tubule region and the hindgut allows metabolic wastes to be cleared from the hemolymph without the significant loss of water. From there the primary urine passes backward through the hindgut and rectum, where it is modified by continued resorption by the rectal glands to produce a **secondary urine** that is expelled through the anus. Compared to humans, where the filtration process treats the entire extracellular volume about 12 times a day, the Malpighian tubules and rectum of the mosquito can filter its extracellular volume up to 200 times a day. The Malpighian tubule cells of *Rhodnius* can each secrete a volume of fluid equivalent to their own cell volume every 15 seconds.

MECHANISM OF MALPIGHIAN TUBULE SECRETION

Early ideas regarding the mechanism of primary urine formation were based on the experimental techniques developed by **Ramsay**. Preparations of Malphigian tubules were bathed in insect Ringer solution, with the open end of the tubule that normally discharges into the gut placed into a layer of oil above the Ringer solution with a silk ligature (Figure 8.11). Fluid flowing through the tubule gathered in an aqueous droplet that then could be measured and collected within the oil. By analyzing the primary urine formed in these droplets, it was discovered that it was isosmotic with the hemolymph, but with potassium concentrations up to 20 times higher. From these initial experiments, the mechanism involving the active transport of potassium into the lumen against an

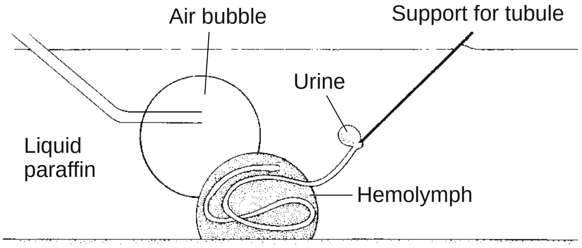

FIGURE 8.11. The bioassay of Malpighian tubule function devised by Ramsay. The excised tubule is immersed in paraffin within a drop of hemolymph and a bubble of air to allow the tubule cells to respire. As the tubule produces wastes, a drop of urine forms at its end. Because the urine is insoluble in paraffin, its diameter can be measured as an estimate of Malpighian tubule activity. From Ramsay (1954). Reprinted with permission.

electrochemical gradient was established. Because the primary urine is isosmotic with the hemolymph, the transport of water appeared to follow the potassium and create the flow of urine within the tubule.

The driving force for the movement of ions results from a **vacuolar-proton-adenosine triphosphatase** (V-ATPase), also known as a **proton pump**, that regulates the concentration of protons. This transduction of ATP hydrolysis into a current of protons, in the absence of any other ions, energizes the cell membranes of the tubules. The translocation of protons into the tubule lumen also plays a key role in the regulation of pH and the acidification of cell compartments. Protons transported into the lumen return to the cell by way of a cation-H^+ exchanger that promotes the movement of sodium or potassium from the cell into the lumen and that drives the process of fluid and waste excretion. The protons secreted into the extracellular region of the brush border return in exchange for Na^+ and K^+. Thus, in addition to the proton pump located on the cell membrane, there are antiport transport processes that carry ions including K^+, Na^+, and H^+ in opposite directions. Na/K ATPases actively transport these ions from the hemolymph, and ion channels allow chloride ions to be transported passively. Although phytophagous insects primarily transport potassium, hematophagous insects such as the mosquito transport sodium and chloride, and the blood-sucking hemipteran *Rhodnius* transports both sodium and potassium. The V-ATPase accomplishes the secretion of all these ions by driving the secretion of sodium or potassium through the antiport ion exchangers and setting up a gradient for the passive movement of small hemolymph solutes into the tubule lumen. Water follows the major ions and the solutes in the hemolymph diffuse via a **paracellular route** between the cells. Active transport, following a **transcellular route**, occurs for some toxins and metabolites, including uric acid (Figure 8.12). This active transport mechanism is located at the plasma membrane of the brush border. Low-molecular-weight substances move through the tubule cell by passive diffusion, but several larger, toxic compounds, such as plant alkaloids, are removed from the hemolymph by an active transport. Organic anions are also transported by tubule cells, allowing them to dispose of insecticides and other unwanted toxic compounds.

The movement of water and other solutes is driven by a concentration gradient and does not require energy, but it does require the presence of selective channels called **aquaporins**. Aquaporins are members of a large family of water channel proteins whose homologs have been found in microorganisms, plants, invertebrates, and mammals. Eleven mammalian aquaporins have been identified so far, and defects in aquaporin genes have been associated with defects in organismal water homeostasis. At least six different aquaporins have also been identified in insects, and as in mammals, their diversity may reflect their abilities to transport glycerol, cations, or anions. They have been found in the cells that make up organs such as the foregut, midgut, and stellate cells of the Malpighian

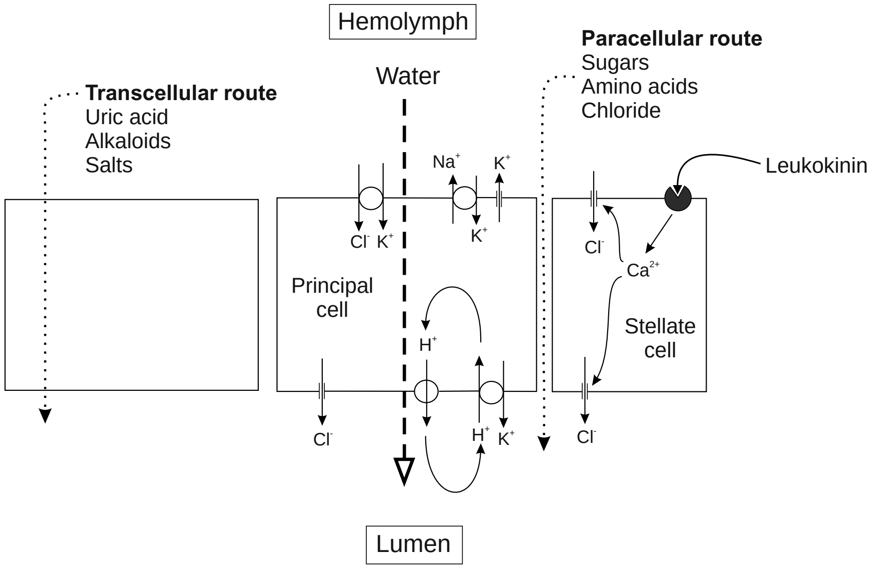

FIGURE 8.12. The transport of substances through the Malpighian tubule cells. The major ion movements result from the action of the V-ATPase that moves protons and energizes the cell membrane. Na/K ATPases actively transport ions from the hemolymph into the lumen, with ion channels allowing some passive transport. Some components move into the lumen by a transcellular route through the cells, while others move by a paracellular route between cells. Chloride ions move through stellate cells when the cells are stimulated by leucokinin. Adapted from Wang et al. (2004). Reprinted with permission.

tubules. Their expression and presence in Malpighian tubule cells are induced by leucokinin and 5-hydroxytryptamine.

HINDGUT AND RECTUM

If insects used the primary urine from the Malpighian tubules as their major excretory product, they would soon be depleted of potassium and water. However, superimposed on the excretion by Malpighian tubules is a second system involving the rectum of the hindgut that recovers most of the ions and water, adjusting the excretory product so that it achieves the necessary osmoregulatory balance for the insect and excretes the secondary urine.

The rectum comprises the enlarged posterior-most section of the hindgut, often containing specialized structures called **papillae** or **rectal pads** that are enlarged epithelial cells. Unlike those of the Malpighian tubules, the cells of the rectum lack the infoldings of the basal membrane, but they do contain a brush

border underneath the cuticle that faces the rectal lumen. Derived from the invagination of epidermal cells, the rectal cells have a cuticular lining on the lumen side that is shed at each molt. Some insects show little in the way of morphological differences that distinguish these rectal pads, and development of the rectum ranges from no modifications in some insects such as *Drosophila* to the specialized rectal pads that are present in many orthopterans and coleopterans. Fluid-feeding insects that do not resorb the fluid in their primary urine may not have rectal pads present. The extent to which the recovery of water and ions occurs is determined by the extent to which water must be resorbed and is a function of environmental and physiological conditions. Insects living under more xeric conditions, such as flour beetles, must resorb more of the water in the hindgut than those living under moister conditions. The rate of this rectal absorption is not constant. *Manduca sexta* larvae are able to maintain a steady state and regulate the water content of their bodies based on the water content of the food they ingest by varying the rate of rectal resorption.

Because the pH of the rectum is often more acidic than the rest of the hindgut and the solubility of uric acid is considerably reduced under acidic conditions, the acidity in the hindgut precipitates uric acid and allows its excretion to occur in the absence of much water. With its cuticular lining, the rectal epithelium is able to act as a molecular sieve that restricts the passage of larger molecules through the cells. Toxic wastes are retained in the rectal lumen by this lining and are excreted with little loss of water.

The rectum works in an opposite manner to that of the Malpighian tubules. It transports water and ions from the material within the gut lumen into the hemolymph. An electrogenic Cl^- pump on the lumen side of the cell membrane that is not coupled to any other ions drives the resorption process, moving Cl^- from the gut lumen into the hemolymph. An Na/K-ATPase mediates the transport of sodium into the hemolymph and generates a positive electrical potential on the hemolymph side of the membrane; it also drives fluid transport. Chloride exits the cell passively into the hemolymph through cAMP-stimulated channels, and potassium also follows passively by an electrical coupling with potassium channels (Figure 8.13).

CRYPTONEPHRIDIAL SYSTEM

In some insects, including tenebrionid beetles that live under extremely dry conditions, the ends of the Malpighian tubules do not lie free in the hemocele. Instead, the terminal segments of the tubules are closely associated with the wall of the rectum in what is called a **cryptonephridial complex** (Figure 8.14). The hidden nature of the tubules, surrounded by a perinephric membrane and creating a special chamber, the **perinephric space**, gives the complex its name. The complex is found in most lepidopteran larvae and many coleopterans. The

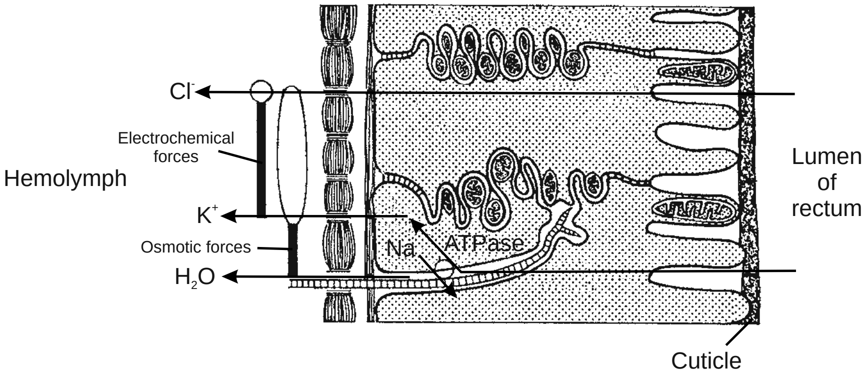

FIGURE 8.13. A rectal cell and its ion transport. From Hevert (1984). Reprinted with permission.

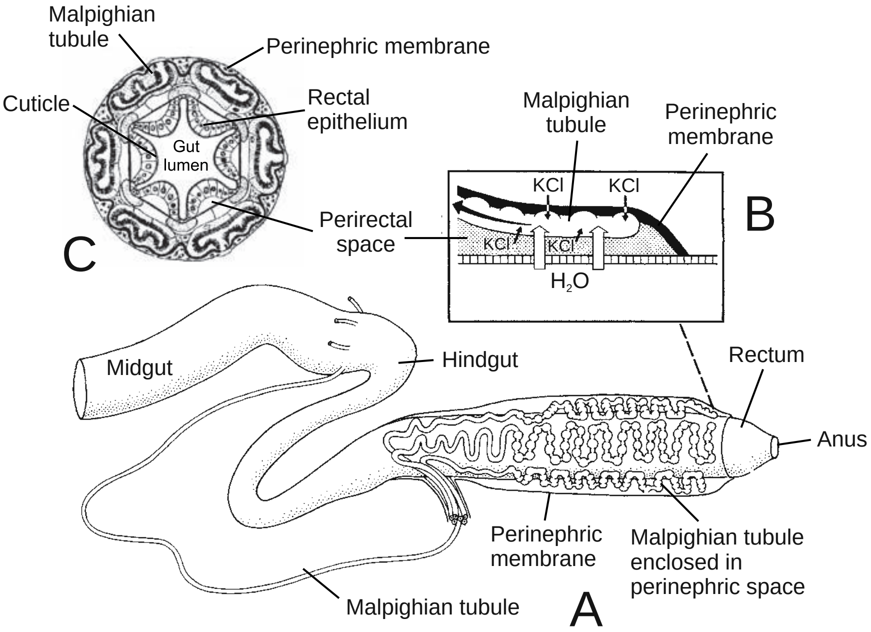

FIGURE 8.14. A. A cryptonephridial complex, showing the close association of the Malpighian tubules with the rectum to serve as a more efficient mechanism of water recovery. B. The inset shows the movement of water and KCl into the tubules from the hindgut and hemolymph, respectively. C. A cross section of the complex. From Bradley (1985). Reprinted with permission.

cryptonephridial complex performs two functions: it resorbs water from the hindgut very efficiently, and in some insects it is able to absorb atmospheric water from the humidity in the hindgut. Both functions rely on the ability of the complex to maintain osmotic gradients through the transport of potassium by the tubules. The tubule cells take up K^+, Na^+, and H^+ ions, creating a sufficiently elevated osmotic pressure for water to be drawn from the rectal lumen into the perinephric space and then into the tubules, where water and some ions are resorbed into general circulation. In other insects, such as the booklice or Psocoptera, atmospheric moisture is absorbed through the specialized hypopharyx within the cibarium at the anterior region of the alimentary canal.

FILTER CHAMBER

In some Homopterans that feed exclusively on plant juices containing low concentrations of nutrients, the digestive tract forms an arrangement known as a **filter chamber**. In this arrangement, the anterior and posterior regions of the midgut come into close contact, sometimes also involving the proximal ends of the Malpighian tubules, and are surrounded by a sac that comprises thin epithelial cells (Figure 8.15). The large amount of fluid acquired during feeding is concentrated and eliminated more quickly by transfer directly from the anterior region of the midgut to the posterior region without being absorbed into and diluting the hemolymph. Potassium is actively secreted into the tubules and posterior midgut, drawing water from the anterior midgut. Much of this fluid containing dilute concentrations of amino acids and sugars is ultimately excreted as "honeydew."

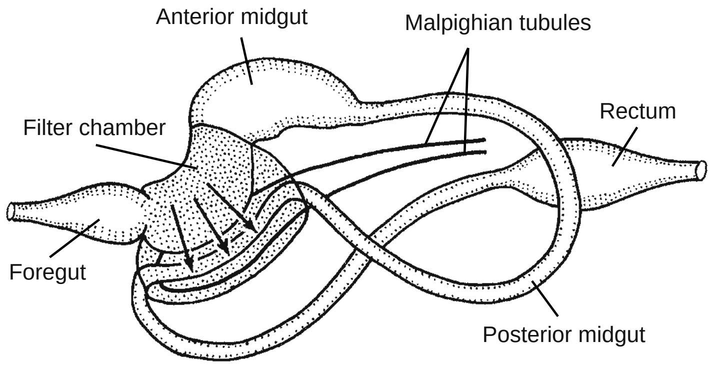

FIGURE 8.15. A filter chamber, in which the anterior and posterior midgut regions are in close contact so that water can be diverted directly to the hindgut without diluting the midgut.

HORMONAL CONTROL OF EXCRETION AND OSMOREGULATION

The Malpighian tubules have no nervous innervations, so the regulation of fluid transport must occur through the action of hormones carried throughout the hemolymph. The insect excretory system is designed to conserve water by maintaining a low rate of excretion under most conditions, but there are sound reasons for accelerating it at various times of the life cycle. For example, as a result of ingesting close to their own weight in grass each day, locusts must rid themselves of the excess water present in the food to prevent their hemolymph from being diluted. Freshwater insect larvae must regulate their hemolymph volume to prevent it from becoming diluted. Other fluid feeders, such as *Rhodnius*, that ingest large volumes of blood during each stadium must process the liquid portion of the diet quickly so as not to overwhelm the circulatory system with water. Insects that must make the transition from a terrestrial phase to a flying phase, such as lepidopterans that metamorphose from a pupa to an adult, undergo an enhanced loss of water to make them better able to fly. Newly emerged *Pieris brassicae* adults thus lose about 40% of their body weight within 3 h of emergence. Obviously, this rate of water loss is reserved for special times during the life cycle and could not continue indefinitely. Surprisingly, there are also many terrestrial insects that live most of their lives under xeric conditions that also employ diuretic hormones. The Malpighian tubules of the locust are stimulated by a diuretic hormone during flight, accompanied by increased absorption by the rectum. In spite of the environment's demands for efficient water conservation, Namib Desert beetles also utilize a diuretic hormone. In both these cases, the increased turnover from hormone activation results in a more rapid clearance of the hemolymph of metabolic wastes without the loss of water because of the efficiency of the rectum in recovering the fluids.

There are many targets for which hormones may modulate excretory rates. The longitudinal muscles that run the length of the tubules and cause them to writhe about in the hemocele have no nervous innervations but are activated hormonally by diuretic and myotropic hormones in the hemolymph. Increased writhing by the tubules of both the stick insect and American cockroach are stimulated by 5-hydroxytryptamine, and myokinins and corticotrophin-releasing factor-related peptides increase writhing in crickets and locusts. These increased writhing movements may augment the circulation of hemolymph over the tubule walls and facilitate the movement of primary urine within the lumen.

The class of hormones known as **diuretic hormones** act on the Malpighian tubules to increase the processing of fluids through the excretory system. Their release at specific times causes an increase in the rate of primary urine production. The Ramsay assay (Figure 8.11) using isolated tubule preparations has served as an excellent bioassay and an indication of this increased rate. By adding suspected diuretic hormones to the drop of hemolymph in which the tubules

are bathed, it is possible to record increases in the production of primary urine. These increases can be dramatic: the mosquito, *Aedes aegypti*, produces a diuretic hormone after blood ingestion that causes the loss of more than 40% of the water in the blood meal within 2 h of feeding. The diuretic hormone in *Rhodnius* can increase the secretion rates of Malpighian tubules by up to 1000 times soon after a blood meal.

The diuretic neuropeptides that have been isolated from insects fall into one of two hormone families: the **corticotropin-releasing factor** (CRF)–related peptides and the insect **kinins**. The CRF-related family of hormones is so named because of its similarity to this family of vertebrate peptides. Their structures are well conserved in those species that are known to have diverged about 300 million years ago and may be present in all insects. The insect kinins were first identified from the cockroach, *Leucophaea maderae*, and caused the contraction of hindgut preparations. They are named according to the genus from which they were isolated: **leucokinins** from the cockroach, *Leucophaea maderae*; **culekinins** from *Culex*, mosquitoes; **achetakinins** from the cricket, *Acheta domesticus*; and **locustakinins** from the locust, *Locusta migratoria*.

The first CRF-related diuretic peptide was isolated from head extracts of larval *Manduca sexta*. The *Manduca*-diuretic hormone (*Manduca*-DH) consists of 41 amino acids and has approximately 30% sequence homology with the CRF family of vertebrate peptides. It stimulates production of cAMP as a second messenger and the subsequent fluid secretion in isolated tubules. Another diuretic peptide, *Manduca*-DPII, was isolated from *Manduca* adults and is only 30 residues in length. Other CRF-related peptides have been isolated from the mealworm, *Tenebrio molitor*; the house fly, *Musca domestica*; the locust, *Locusta migratoria*; and the cockroach, *Periplaneta americana*. The *Locusta*-DH is synthesized in the pars intercerebralis of the brain and transported to the storage lobe of the corpus cardiacum where it is released. In the adult female mosquito, *Aedes aegypti*, a CRF-like **mosquito natriuretic peptide** causes the increased diuresis that is necessary after a blood meal. Malpighian tubule activity is increased by the ability of these CRF-related peptides to open sodium channels. A redistribution of the β-actin cytoskeleton within the tubules has been correlated with increased intracellular cAMP and heightened urine flow and may be responsible for the changes in microvillar growth and intracellular fluid transport.

The insect kinins also stimulate fluid secretion by isolated Malpighian tubules. Unlike the CRF-related peptides, the kinins have no effect on cyclic AMP but instead increase intracellular calcium, which serves as a second messenger in stellate tubule cells to regulate chloride transport. The peptides are produced by neurosecretory cells of the pars intercerebralis and are transported to the storage lobes of the corpus cardiacum via the NCC I where they are released. The kinins are small peptides of between 6 and 14 amino acids that were first characterized as myotropins before their diuretic function was discovered. In contrast to the CRF-related peptides, the insect kinins cause sodium transport to decline

and potassium transport to increase. Specific kinin-binding sites have been identified on the Malpighian tubules of crickets. Cockroach leucokinins are active on the Malpighian tubules of mosquitoes, crickets, houseflies, corn earworms, and *Drosophila.*

The importance of excretion to small terrestrial animals like insects is reflected in the complexity of the systems that regulate it. The presence of CRF-related peptides and the kinins is not mutually exclusive, and both may be active in a particular species, perhaps acting in a synergistic or modulatory fashion to precisely control Malpighian tubule function and the specific ions to be transported. In the blood-sucking bug, *Rhodnius prolixus*, there are two factors that regulate diuresis: a peptide diuretic hormone and **5-hydroxytryptamine** (5-HT), both of which act synergistically to stimulate fluid secretion by the tubules after a blood meal. The 5-HT inhibits the Na/K ATPase, and the resulting inhibition of sodium moving into the hemolymph causes it to instead move into the tubule lumen. 5-HT is active in *Manduca sexta* and adult *Aedes aegypti*, and in blood-fed *Rhodnius* it induces the expression of aquaporin-related proteins. In the mosquito, 5-HT also abolishes hindgut peristalsis. Another peptide, insect **cardioacceleratory peptide 2b** (CAP_{2b}), stimulates the production of the enzyme **nitric oxide synthase** (NOS) that is responsible for the synthesis of the signaling gas **nitric oxide** (NO), which stimulates fluid secretion in the tubules by stimulating the enzymatic pathways in principal cells for cGMP production. CAP_{2b}, involved in regulating the heartbeat of *Manduca* during the wing inflation following adult emergence, has also been described as an antidiuretic hormone in *Rhodnius*, with cGMP as a second messenger, but in this case without nitric oxide.

The **CAPA** family of neuropeptides is also involved in NO/cGMP signaling. Products of the *capability* gene in *Drosophila*, CAPA-1 and CAPA-2, are closely related to CAP_{2b} and induce diuresis in *Drosophila* and *Anopheles gambiae* by stimulating NOS activity and increasing cGMP levels. Calcitonin is known to participate in calcium and phosphorus metabolism in mammals, and several **calcitonin-like peptides** stimulate the secretion by principal cells of the Malpighian tubules of cockroaches and locusts. The monoamine, **tyramine**, may act on the principal cells in *Drosophila* to increase chloride ion conductance and urine production.

The absence or inactivation of diuretic hormones generally terminates the accelerated diuresis by Malpighian tubules. Fluid loss can also be prevented by increasing the resorption by the hindgut. **Antidiuretic hormones** act on the hindgut to increase its resorption of the primary urine that is produced by the Malpighian tubules. The **chloride transport stimulating hormone** (CTSH) is a peptide of approximately 8 kDa and in the grasshopper, *Schistocerca,* is produced in the pars intercerebralis of the brain and transported to storage and glandular lobes of the corpus cardiacum via the NCC I. It activates the electrogenic uptake of chloride and opens potassium channels in rectal cells using cAMP as a second messenger. As with diuretic hormone, CTSH is released after feeding in the

locust, and the combination of the two hormones may increase fluid cycling to enhance the clearance of the hemolymph without altering the excretory rate.

Another antidiuretic hormone acts on the ileum of the hindgut to increase its rate of resorption. The **ion transport peptide** (ITP) has a molecular weight that is between 7.7 and 8.7 kDa, and it is located in the storage lobe of the CC. It is a member of a family of crustacean neuropeptides that includes a crab hyperglycemic hormone and a lobster molt-inhibiting hormone. It acts with cyclic AMP as a second messenger, causing increases in sodium absorption, entry of chloride via an electrogenic pump, and ammonia secretion into the lumen.

In several coleopterans, antidiuretic factors that act on the Malpighian tubules, rather than the hindgut, have been identified. The antidiuretic factors **ADFa** and **ADFb** stimulate cGMP and consequently inhibit fluid production by the tubules as a result of their action to reduce cAMP levels. In blood-feeding *Rhodnius*, the peptide **Mas-CAPA-1** stimulates the intracellular second messenger cGMP that degrades cAMP and reduces its usual secretory actions. These antidiuretic factors might be targets for insect control, with suitable antagonists able to impair the ability of insect pests to survive in dry environments.

STORAGE EXCRETION

One strategy for dealing with wastes is to sequester them within the body rather than eliminate them. Because uric acid is so insoluble, it can be easily stored without it interacting with other physiological processes. Some cockroaches accumulate up to 10% of their dry weight in uric acid stored in specialized urate cells in the fat body, which can be utilized during periods of dietary stress. However, because the degradation of the stored uric acid occurs by microbial symbionts in specialized cells of the fat body, this may not be a good example of strict storage excretion. In this case, the net amount of uric acid in the fat body may simply be a result of the balance between synthesis by the insect and degradation by the symbionts.

In some Lepidoptera, the fat body shifts from excretion of uric acid to its storage during the last larval instar. With the onset of wandering, normal uric acid excretion is terminated and uric acid begins to be accumulated within the insect. This uric acid is stored in the fat body during the pupal stage but is transported to the rectum for elimination shortly before adult eclosion. The switch from elimination to storage is the result of the exposure to 20 HE in the absence of JH. The switch back to transport results from declining levels of 20 HE after the molting peak. The males of some cockroach species store as much as 5% of their total live weight in uric acid maintained in the male accessory glands. The uric acid is incorporated into the spermatophore and used by the female after mating as a nitrogen source for the development of the ootheca and eggs. In the tropical cockroach, *Xestoblatta hamata*, the male excretes this

urate and offers it to the female before copulation. The status of the female's dietary nitrogen level determines how much of this secretion is fed upon, and the nitrogen is incorporated into her eggs. This nuptial gift shortens the time from mating to oviposition.

Other waste products may be utilized in other ways. Some locusts are able to feed on plants containing high levels of phenols that serve as feeding deterrents for most other species. The locust incorporates these phenols into the cuticle by using them to cross-link cuticular proteins that usually are linked by tyrosine and saving the amino acid for other functions. The coloration of some scarab beetles results from the deposition of uric acid in the cuticle, and *Pieris* butterflies deposit pterins, which are end products of nitrogen metabolism, in their cuticles.

OTHER FUNCTIONS OF THE MALPIGHIAN TUBULES

The Malpighian tubules of some insects synthesize silk. In the larvae of the lacewing, *Chrysopa*, some of the tubule cells thicken and produce the silk used to construct the pupal cocoon. The tubules of larval ant lions also produce silk that is stored in a rectal sac and used during pupation. Larval spittle bugs produce the spittle in which they live from their Malpighian tubules. The calcium used to fortify the puparium of the face fly, *Musca autumnalis*, is transferred from the tubules to the cuticle. Microfilariae of *Dirofilaria immitis*, the nematode responsible for dog heartworm, are ingested by adult mosquitoes and develop in the distal cells of their Malpighian tubules. Large numbers of parasites within the tubules can kill the mosquitoes.

Immune-responsive sites in insects consist largely of the fat body and hemocytes, but other tissues, including epidermal cells, are additionally capable of producing immune factors. The Malpighian tubules, which are also epidermal and among the first to be exposed to molecules in the hemolymph that indicate an immune challenge is under way, additionally express the genes in the Imd pathway that deal with the elimination of gram-negative bacteria. They may thus provide the insect with one of the initial signals that an infection has occurred.

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CHAPTER 9

Respiratory Systems

Although there are many striking similarities between the physiological systems of vertebrates and those of insects, one conspicuous difference is in the way that oxygen is brought to the cells. This difference was sufficient for Aristotle, in 350 B.C.E., to characterize insects as terrestrial animals that had no requirement to inhale. It was Malpighi in 1669 who first identified the series of branching tubes that bring oxygen directly to tissues (Figure 9.1), a departure from the system in vertebrates where air is regularly and often visibly drawn into the body to oxygenate body fluids that are then circulated to all cells.

BRINGING OXYGEN TO INSECT CELLS

The exchange of materials with the environment is a fundamental requirement for living cells. Oxygen and nutrients must be taken up and metabolic wastes must be discarded. Because all cells are bounded by a cell membrane and consist largely of water, every cell must be bathed in an aqueous medium, and the materials to be transferred must be dissolved in water. However, membranes that allow the larger oxygen molecule to enter through it also allow the smaller water molecule to exit. Thus, the ability to take up oxygen through a membrane is

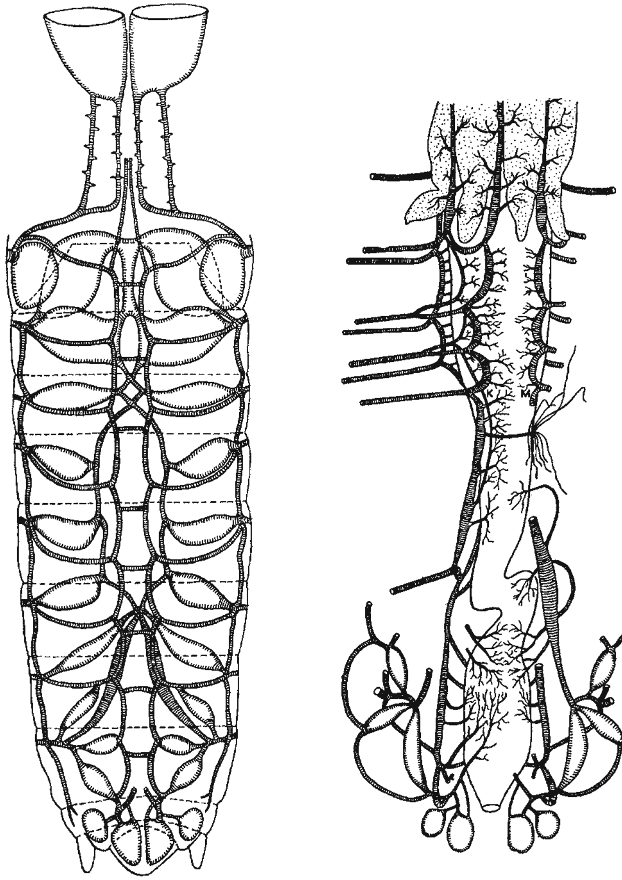

FIGURE 9.1. Views of the locust tracheal system. (*Left*) Dorsal system in abdomen. (*Right*) Tracheae associated with the alimentary canal. From Albrecht (1953). Reprinted with permission.

also associated with the potential loss of water. In small single-celled animals such as protozoa that live in an aqueous medium, the exchange occurs easily over the surface of the cell. For larger multicellular terrestrial animals, where not every cell is exposed to the environment, systems have evolved that bathe each cell in body fluids that are able to mediate this exchange.

The hemolymph of the insect circulatory system is responsible for bathing all cells and allowing them to exchange nutrients and metabolites, but the transport of oxygen to the cells occurs through a tracheal system where external surfaces are invaginated into the body cavity to provide an oxygen pipeline from the outside. Oxygen is far more soluble and diffuses about 10^6 times faster in air than in water, so the use of air in the tracheal system as an internal transport

medium instead of using water not only saves water but is also more efficient. By providing a direct supply of oxygen to their cells, insects have eliminated the reliance on an extensive network of fluids that circulates oxygen as in vertebrates. There are hemoglobin-like respiratory pigments within the hemolymph and tracheoles of some insects, but in general the circulatory system and the blood within it play a very minor role in gas exchange.

Although the tracheal system provides an enormous surface area that is permeable to both water and oxygen, water loss by this route is minimal because the system is only open to the outside at the small area that the spiracles present to the environment (Figure 9.2). The volume of the tracheal system in *Hyalophora* pupae averages 48 $\mu l\, g^{-1}$ of body weight and consists of about 10,000 interconnected tubes in *Drosophila* larvae. This extensive system is effective in supplying oxygen when required. Compared to rates at rest, the rate of oxygen uptake during insect flight increases from 20 to 100 times with no measurable oxygen debt, whereas in vertebrates the maximum increase during physical activity is only about 12-fold. Hummingbirds, known for their high metabolic rates during flight, consume about 2 $ml\, g^{-1} min^{-1}$ of oxygen, but hovering by the worker honeybee consumes three times this rate.

There are some drawbacks to using tracheae for respiration. It has been speculated that the dynamics of the tracheal system have kept insects out of the most abundant habitat on the planet, the oceans. Oxygen travels through the insect respiratory system by diffusion, and because the extreme pressures at lower

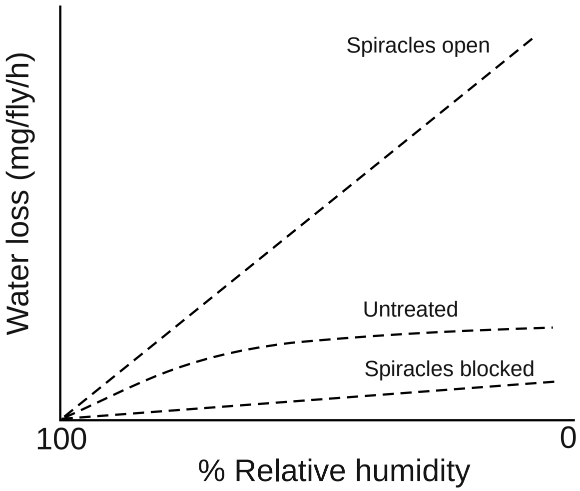

FIGURE 9.2. The effectiveness of the spiracles in minimizing water loss in the fly. As the relative humidity decreases, the water lost by untreated insects and those with blocked spiracles levels off. When the spiracles remain open in a carbon dioxide atmosphere, water loss is significant.

depths would prevent the tracheal system from operating this way, insects were never able to colonize the harborages that are deep at the ocean floor. In contrast are the gill-bearing crustaceans, a group of arthropods that have successfully carved out an aquatic niche in part because their respiratory system operates at greater depths. The insects are thus relegated to the upper ranges of the oceans, where in the absence of hiding places, they are more likely be preyed upon by larger aquatic organisms and have never become established.

The reliance on a tracheal system also limits the maximum body size that insects can attain. In small insects, simple diffusion of oxygen is sufficient. In larger insects, this diffusion may be supplemented by the ventilation of tracheal sacs, but even this has its limits. The safety margin for oxygen under which the honeybee operates is exceeded at altitudes above 3000 m because of limitations in the convective delivery of oxygen to the flight muscles. Tracheal mites pose another respiratory problem: the safety margin for oxygen delivery in bees is reduced by the infestation of these parasites in the tracheal system. *Drosophila* larvae that are reared at reduced oxygen concentrations emerge as adults with a lower body mass and increased developmental times than those reared at normal atmospheric levels (Figure 9.3). The effect of low oxygen concentration begins to show up during pupal development.

The fossil record indicates that now-extinct insects from the Carboniferous, approximately 300 million years ago, were often significantly larger than present-day species, with dragonfly wingspans recorded to be as large as 70 cm. These giants evolved under different atmospheric conditions than those that are present today. An atmospheric oxygen concentration of as high as 35%, compared to the present-day 21%, undoubtedly had profound implications for the size that insects could attain as a result of the relative ease of distributing oxygen to their tissues. Increases in atmospheric oxygen were also probably responsible for the evolutionary terrestrialization of both insects and vertebrates that occurred 345 million years ago.

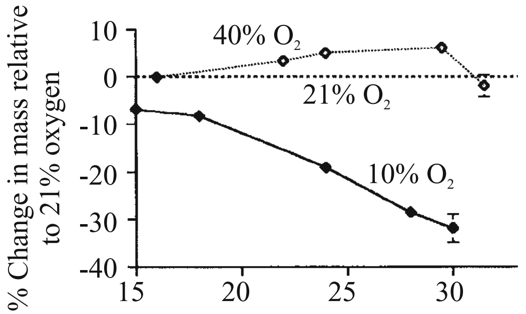

FIGURE 9.3. The change in body mass when *Drosophila* were reared at three oxygen concentrations compared to that at 21% oxygen. From Frazier et al. (2001). Reprinted with permission.

THE TRACHEAL SYSTEM

Development of the larval tracheal system of *Drosophila* begins with the invagination of epithelial cell clusters to form 20 epithelial sacs, 10 on each side of the embryo. Each sac is destined to become the trunk of the tracheal system and acquire their tracheal identity beginning at about 4 h after the egg is laid. The approximately 80 cells in each of these sacs then begin a synchronized outgrowth of secondary and terminal branches that results from a developmental program that is modified by the need for oxygen by local tissues, involving cell migration, cell intercalation, the formation of new cell junctions, and changes in cell shape and size to produce a series of interconnected tubes (Figure 9.4).

The outgrowth of primary tracheal branches is induced by the production of the **branchless** (**Bnl**) protein by clumps of cells that surround each tracheal sac (Figure 9.5). Branchless is a homolog of the **mammalian fibroblast growth**

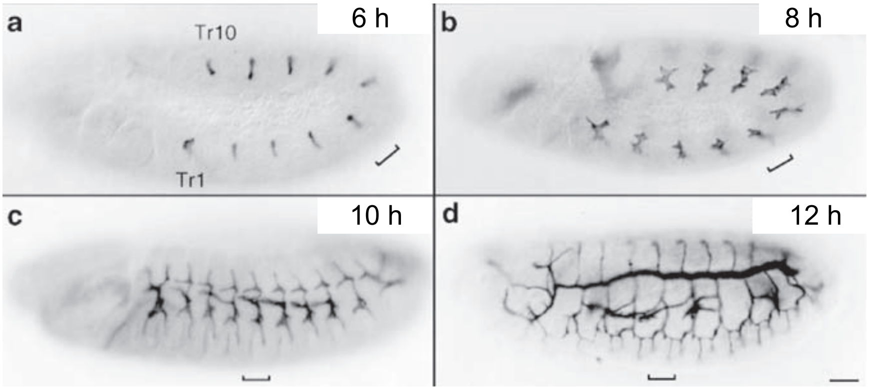

FIGURE 9.4. The growth of tracheal branches during *Drosophila* development. From Ghabrial et al. (2003). Reprinted with permission.

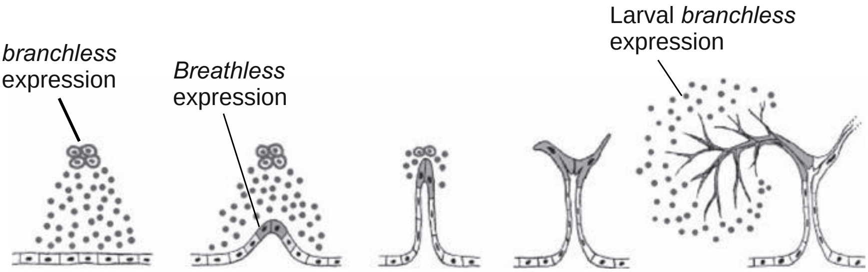

FIGURE 9.5. The outgrowth of tracheal branches in response to the expression of *branchless* by cells that surround the tracheal sacs where primary branches will bud. Target tracheal cells respond by expressing the receptor encoded by the *Breathless* gene.

factor (**FGF**), which in vertebrates directs cells to divide, differentiate, or migrate, and as the first FGF family member to be identified in invertebrates, it may therefore be a universal patterning molecule in both insects and vertebrates. The expression of the *branchless* (*bnl*) gene by these cells activates the FGF receptor **breathless** (**Btl**) in target cells that is necessary for signal transduction. Several other signaling systems are responsible for the branching subsets that are created by cell rearrangements during branch morphogenesis. Body cells that undergo hypoxia express Bnl, and nearby tracheal branches respond to this chemotactic factor by growing new tubes toward the source. When the tracheal system is extensively remodeled during the later metamorphoses to the pupa and adult in order to supply tissues with an appropriate supply of oxygen, FGF-like molecules again cause the proliferation of certain cell types in the imaginal discs that will become adult tissues.

Because the tracheal system arises as invaginations of the epidermis, it is consequently surrounded by epidermal cells that secrete their cuticles inward toward the lumen (Figure 9.6). The tracheal cuticle typically consists of a chitinous endocuticle and the epicuticle, which is thrown into a spiral fold called the **taenidium** (Figure 9.7). These taenidia, which may contain chitin, provide the rigidity that prevents the tracheal tubes from lateral collapse because of changes in air pressure. The size of the taenidia is proportional to the diameter of the tubes, ranging from a few micrometers in trunks to several nanometers in tracheoles. As a consequence of being epidermal in origin, the tracheal cells produce a tracheal cuticle that is homologous with that of the integument. The tracheal cuticle molts along with the integumental cuticle after first digesting the old endocuticle and then shedding the thin epicuticle that remains. Unlike the epidermal epicuticle, the tracheal epicuticle in most insects lacks the cement and wax layers. A wax layer has been identified in the tracheal cuticle of some lepidopteran larvae.

The tracheal tubes open to the outside at the **spiracle**, sometimes surrounded by a circular sclerite known as the **peritreme** (Figure 9.8). An **atrium** may be

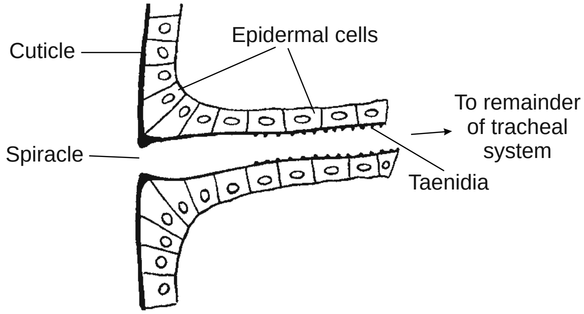

FIGURE 9.6. The development of the tracheal system by the ectodermal invaginations that occur during embryogenesis. From Snodgrass (1935). Reprinted with permission.

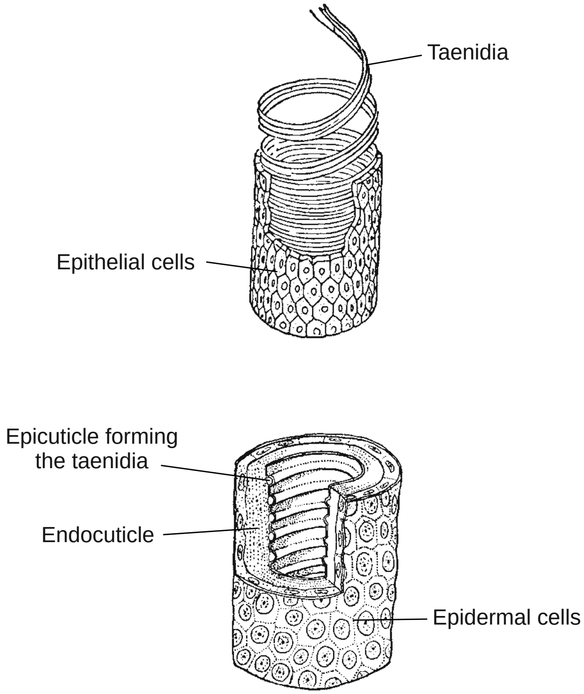

FIGURE 9.7. (*Top*) A tracheal trunk with its taenidial lining. (*Bottom*) The epidermal cells produce the taenidia as part of the epicuticular layer. From Wigglesworth (1965). Reprinted with permission.

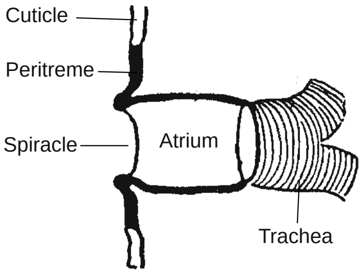

FIGURE 9.8. The opening of the tracheal system at the spiracle. The atrium is an internal cavity just beyond the spiracle that opens to the tracheal system. From Snodgrass (1935). Reprinted with permission.

present, which is an internal cavity lined with hairs to reduce water loss and collect particulates. There is never more than one pair of spiracles per body segment, and the evolutionary trend has been toward the reduction of total numbers of spiracles. Spiracles are never present in the head capsule. The primitive number of spiracles was probably 12 pairs, but present-day insects have no

more than 11, consisting of three thoracic and eight abdominal pairs, a condition found in diplurans. There are fewer spiracles present in other insects.

When all spiracles are present and functional, it is known as the **holopneustic** condition (Figure 9.9). When several pairs have become nonfunctional through evolutionary loss, the condition is referred to as **hemipneustic**. The **propneustic** and **metapneustic** conditions are found in pupal and larval dipterans in which only the first or last abdominal pair is functional. Insects that have no functional spiracles are termed **apneustic**, although they still retain an internal tracheal system and extract oxygen through a cuticle that is modified. With the exception of apterygote insects, the tracheae in most other insects are usually connected by large tracheal trunks to allow even a single spiracle to ventilate the entire system. The tracheal system is completely absent in some primitive collembolans that live in moist environments, and these insects respire directly through the cuticle.

Spiracles in terrestrial pterygotes are often equipped with closing mechanisms that can restrict water loss by shutting off the tracheal system when oxygen uptake is not required (Figure 9.10). There may be cuticular flaps on the outside that cover the spiracle, or there may be internal valves that close off the tracheal system from the spiracular opening. When the valves bear only one closer muscle, the antagonistic action of cuticular elasticity causes them to open when the muscle is relaxed. There may also be an additional opener muscle associated with some spiracles. The muscles that control these valves are innervated from the ganglia of the ventral nerve cord (Figure 9.11), which contain receptors for both carbon dioxide and oxygen. Some spiracles, especially when a single muscle controls their opening, may be affected by high levels of CO_2 that act directly on the muscle to cause it to relax and open the valve by the elastic action of the cuticle. When the hemolymph becomes more concentrated with water loss, the increased concentration of ions that results causes the spiracles to stay closed longer. The accumulation of CO_2 during respiration can also increase the H^+ concentrations, and the consequent change in the pH of body fluids affects the spiracular opening. The ganglia may be affected by low oxygen concentrations in the hemolymph, causing the spiracular valves to initiate fluttering. The spiracles need only remain open for a brief moment to admit air without engendering a significant water loss.

The diameter of tracheae at the spiracle may be several millimeters in large insects, but they branch and grow smaller in diameter until tapering to 1 to 2 μm. At this diameter, the tracheae give rise to **tracheoblasts** that produce the **tracheoles** that mediate the transfer of oxygen to cell mitochondria. Tracheoles taper to approximately 0.1 μm and likewise produce a chitinous cuticle and taenidia, but this cuticle is not shed during the molt. The cuticle of tracheoles is lined with an outer epicuticular layer. The surfaces of the tracheoles lie close to sites of oxygen uptake, but they do not penetrate cell membranes. Tissues are covered with an investment of tracheoles, and based on diffusion constants the

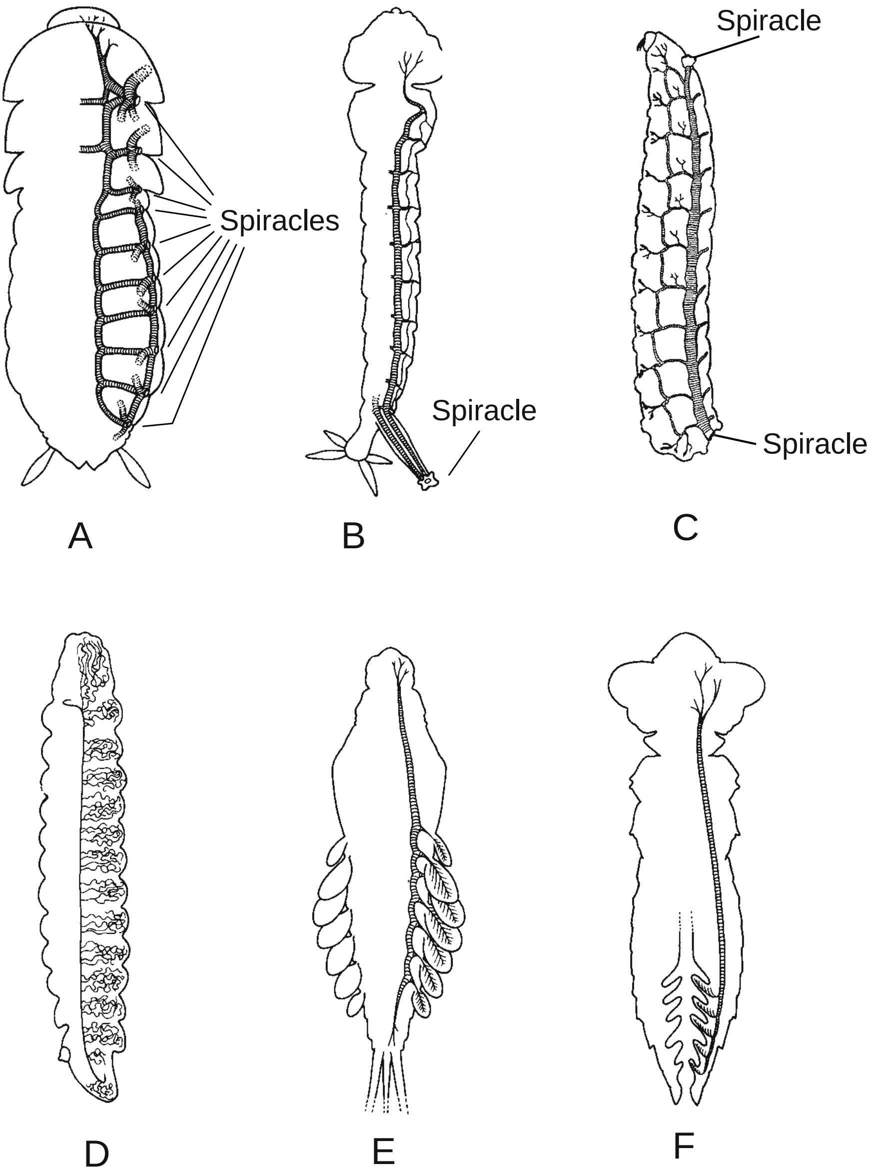

FIGURE 9.9. Spiracular systems. A. The holopneustic arrangement in which all spiracles are open. B. A metapneustic arrangement, with the only open spiracle borne on a siphon tube on the last abdominal segment. C. The hemipneustic configuration with two functional spiracles. D. An apneustic arrangement, with no spiraclular openings but a cuticle heavily invested with tracheoles to permit cuticular respiration. E. An apneustic arrangement with tracheoles concentrated in cuticular flaps. F. An apneustic arrangement with rectal gills, in which oxygen is extracted from the tracheoles that invest the hindgut. From Gullan and Cranston (2000). Reprinted with permission.

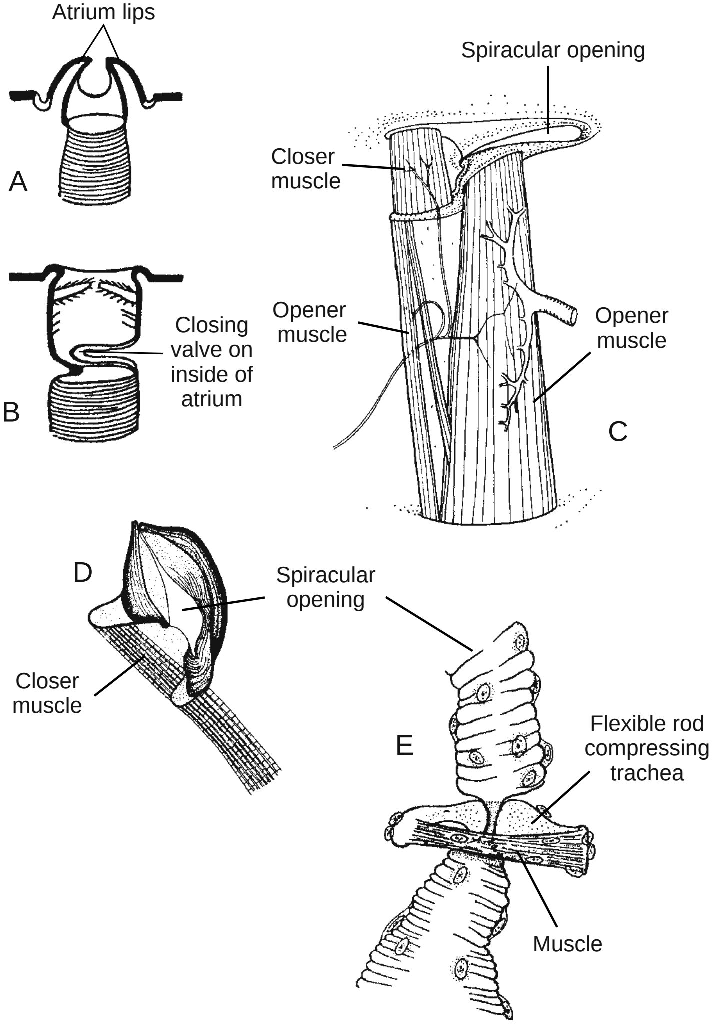

FIGURE 9.10. Mechanisms of spiracular opening and closing. From Miller (1974), Nikama and Khole (1988), and Wigglesworth (1965). Reprinted with permission.

cell mitochondria must ideally be spaced at least 4 to 8 μm away from a tracheole in order to receive a sufficient supply of oxygen (Figure 9.12). In insect flight muscles, the tracheoles may actually be spaced about 2 to 3 μm apart and invaginated into the muscles, but they never actually penetrate the cell membranes to become functionally intracellular. Tracheoles arising from tracheoblasts grow like the nodes on a plant but are not shed during the molt (Figure 9.13). A ring of

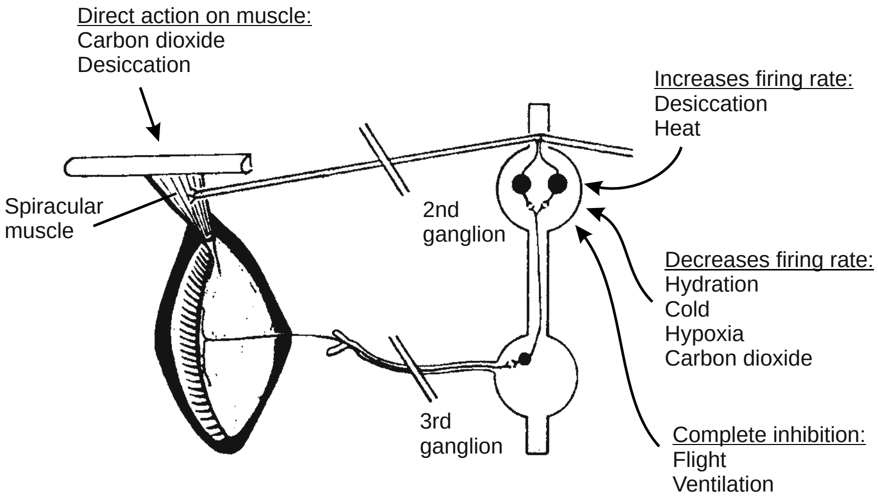

FIGURE 9.11. Nervous innervation of the spiracular closing muscle and the mechanisms that affect its action. From Nikam and Khole (1989). Reprinted with permission.

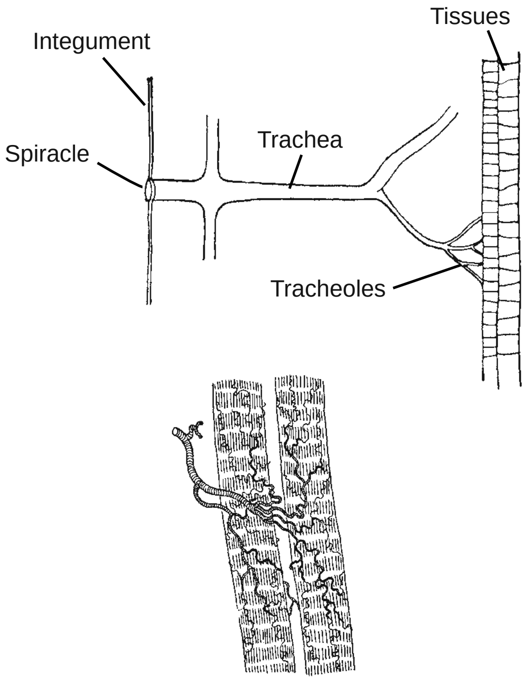

FIGURE 9.12. (*Top*) Trachea branching into tracheoles at the muscle. (*Bottom*) Tracheoles arranged to provide a loose investment around muscle tissue. From Snodgrass (1935). Reprinted with permission.

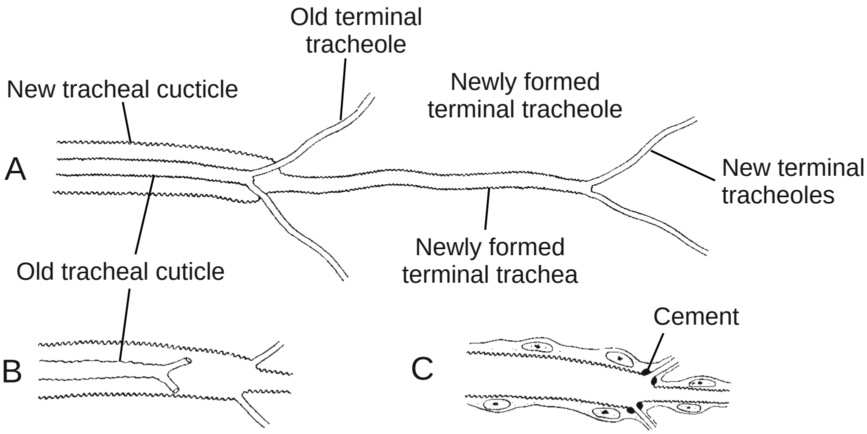

FIGURE 9.13. The mechanism of new tracheal and tracheole growth during a molt. A. After apolysis and deposition of new tracheal cuticle. B. The old tracheal cuticle is detached and pulled out of the tracheal tube. C. Cement produced by the tracheole cells secures them to the old new tracheal cuticle. From Wigglesworth (1981). Reprinted with permission.

cement secures the connections between the old tracheole and the new trachea after growth.

A small amount of liquid may be present in the tracheole at the cell interface if the insect is not respiring actively, but it is drawn into the cell during muscular activity. This movement of liquid appears to be the result of the balance between capillary action pulling out the fluid from cells and the osmotic uptake resulting from the buildup of metabolites within the cell.

Cutaneous respiration occurs in some apterygotes, aquatic insects, and endoparasites. A thin cuticle is invested with an extensive tracheal system that allows the integument to act as a gill to transfer oxygen from the water. Mud-dwelling chironomid larvae that live in oxygen-poor stagnant water supplement their cutaneous respiration with the respiratory pigment **hemoglobin** that is dissolved in the hemolymph. Hemoglobin is also a component of the modified fat body cells in the larvae of the dipteran *Gasterophilus*, which live in the stomach of horses and often are faced with oxygen deficiencies. *Anisops*, an aquatic hemipteran, harbors hemoglobin within tracheal cells that helps maintain buoyancy and allows them to remain submerged for significantly longer. Hemoglobin certainly makes sense in these species that live under hypoxic conditions, but surprisingly, hemoglobin has been identified in other terrestrial insects whose tracheal systems alone were considered to be sufficient. *Drosophila* larvae and adults, the silkworm, *Bombyx mori*, and the honeybee, *Apis mellifera*, express the gene for hemoglobin in cells of their tracheal systems. In these cases, the hemoglobin may serve as a myoglobin-like respiratory molecule that facilitates the transfer and storage of oxygen from the air within the tracheae to the tissues,

or it may function as an oxygen sensor and oxygen donor involved in tracheal growth and nitric oxide synthesis. Its presence in several blood-feeding insects may serve as protection from the oxidative stress that the heme released from hemoglobin digestion might cause.

Hemocyanins are a family of copper-containing respiratory proteins thought to be present only in noninsect arthropod groups such as crustaceans and chelicerates. A related family of proteins, the **hexamerins**, are present in all insects and function only as larval storage proteins because they lack the copper-binding sites that would allow them to bind to oxygen. Hemocyanins are present in primitive stoneflies as respiratory proteins, suggesting that they may have been inherited from the common ancestor of insects and crustaceans and then evolved into storage proteins once the structure of the respiratory system no longer required them.

The tracheal systems of some endoparasitic hymenopterans are filled with fluid during the first instar and only contain air beginning during the second instar. The spiracles are nonfunctional during most of the larval stage until the parasite is ready to leave the host.

MODIFICATIONS THAT INCREASE OXYGEN UPTAKE

The diffusion of oxygen through the tracheal system is adequate enough for a small insect to respire, but larger, more active insects use additional means to supplement this diffusion. Abdominal pumping in some insects ventilates the tracheal system by changing its volume. Muscular contractions of the abdomen change its shape, consequently contracting and expanding the volume of the tracheal tubes within it, thereby allowing them to be ventilated. There are areas of the tracheal trunks that may also be dilated into sacs that have reduced taenidia, thus allowing the trunks to be compressed by changes in hemolymph pressure so that air can be pumped in and out as with a bellows (Figure 9.14). Many larger insects appear to be breathing as they pump their abdomens and thereby change the volume of these tracheal sacs.

When the tracheal sacs are located around the flight muscles, they can be pumped when the insect flies. In flying locusts, the peak demands of the flight muscles for oxygen are better met when the volume of the sacs is automatically increased and decreased by contractions of those muscles during flight. Each muscle is supplied with a primary tracheal trunk and air sac so that the tracheoles are well supplied with oxygen. In the locust, *Schistocerca,* abdominal pumping can ventilate the tracheal system by 40 L/kg/h, but the additional thoracic pumping during flight can increase this rate to 250 L/kg/h. The opening and closing of spiracular valves along the body segments is coordinated to produce a directed flow of air through the tracheal system.

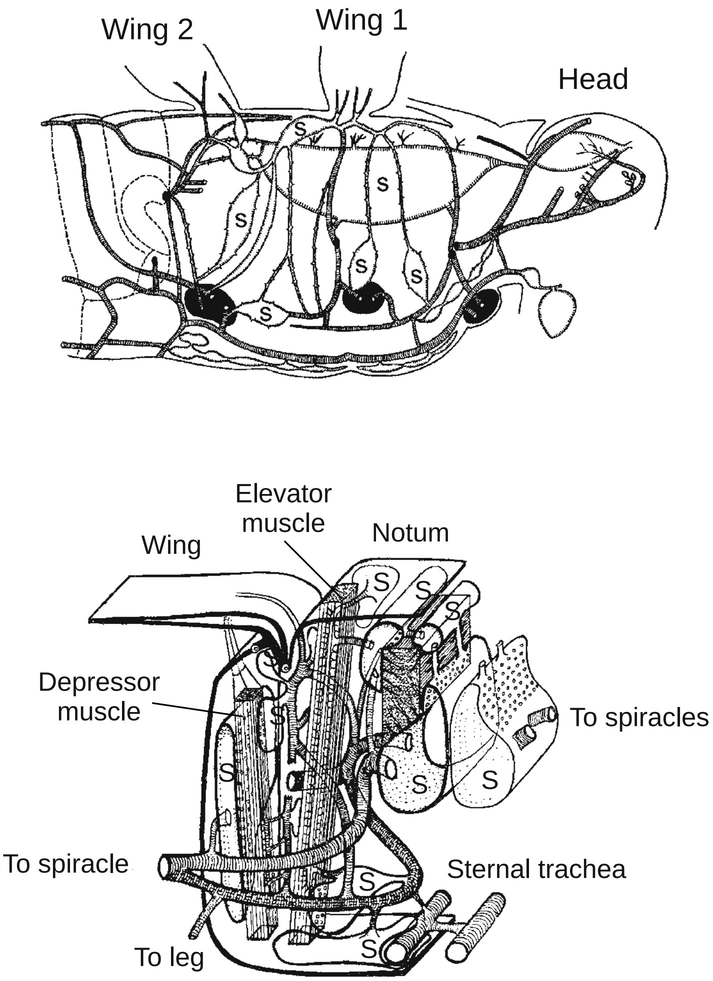

FIGURE 9.14. Tracheal air sacs(s) within the tracheal system can be increased and decreased in volume to pump air through the system. From Albrecht (1953). Reprinted with permission.

The circulatory system can affect the rate at which air enters the tracheal system. The heartbeat, normally directed anteriorly by peristaltic contractions of the dorsal vessel, can reverse its direction and beat backward. During this reversal, hemolymph accumulates in the abdomen, compressing the abdominal air sacs and forcing air out of the tracheal system. At the same time, the thoracic air sacs expand, bringing air into the spiracle that supplies these tracheae. By alternating its flow of hemolymph, the blowfly, *Calliphora*, can modulate the ventilation of the tracheal system (Figure 9.15).

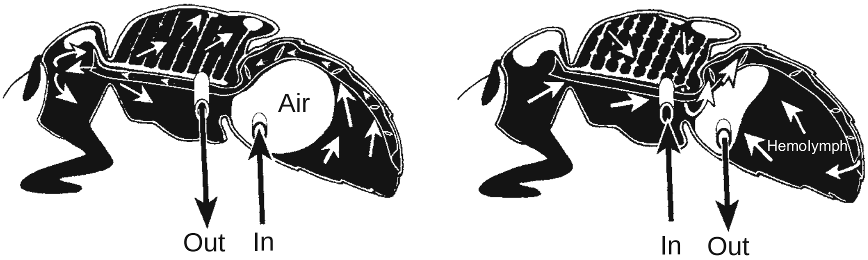

FIGURE 9.15. The regulation of tidal air flow in the tracheal system by the movement of hemolymph. By moving the hemolymph into the thorax or abdomen, it can compress tracheal sacs, causing air to move in or out. From Wasserthal (1996). Reprinted with permission.

NONRESPIRATORY FUNCTIONS OF TRACHEAL SYSTEMS

Spiracular systems may have accessory functions in addition to their role in gas exchange. In the grasshopper, *Romalea*, the tracheal system is modified for the release of defensive secretions. A phenolic compound is produced by a glandular epithelium in the spiracular trunks that is expelled along with air. Similarly, in the cockroaches, *Diploptera punctata* and *Blaberus discoidalis*, defensive secretions are forced out with air pressure through the spiracular openings. In the Madagascar hissing cockroach, *Gromphadorhina portentosa*, the hissing sounds are made when air is expelled through a modified spiracle. The hissing may be used defensively or during mating.

DISCONTINUOUS GAS EXCHANGE

Rather than the continuous ventilation expected by diffusion, a periodic cycle of gas exchange occurs in many insects when they are resting. These cycles are characterized by long periods of spiracular closure, followed by a spiracular flutter and finally an open phase during which levels of CO_2 and O_2 equilibrate with those in the atmosphere (Figure 9.16A). Known as **discontinuous gas exchange cycles**, they were first identified in diapausing moth pupae as a mechanism for reducing water loss during respiration when drinking was not possible.

The pattern of this discontinuous release begins when the spiracles are closed, during which time oxygen is utilized from the air already present in the tracheal system. As the oxygen is consumed, carbon dioxide that is produced is first expressed as increased bicarbonate concentrations in the hemolymph. When the oxygen levels reduce, the spiracular opener muscles relax and the spiracular valves flutter, allowing oxygen to enter the tracheal system. As critical levels of carbon

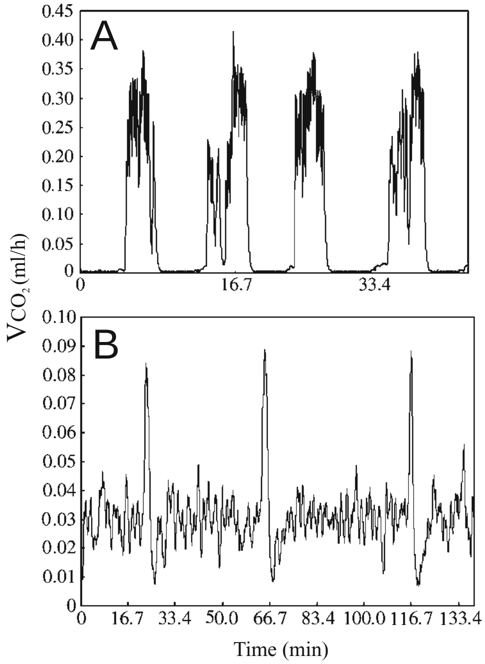

FIGURE 9.16. A. Discontinuous respiration from a cockroach. B. Cyclical respiration from a dragonfly. From Marais et al. (2005). Reprinted with permission.

dioxide accumulate in the tracheae, the spiracles open and allow the gas to escape. Thus, oxygen entry is fairly continuous but the loss of carbon dioxide, along with water, only occurs discontinuously during the brief open phase of the spiracles. Another pattern of CO_2 release is termed **cyclic gas exchange** because it is continuously expelled, but with rhythmic increases and decreases (Figure 9.16B). These cyclic patterns tend to be associated with higher temperatures.

Discontinuous respiration has been identified in more than 100 insect species and may have arisen independently at least five times in arthropods, but the respiratory patterns in one third of the insect orders still remain unexplored, and a meaningful comparative perspective has not been achieved. Rather than being an ancestral feature, it may be adaptive and more related to insect habitats. Although discontinuous respiration has been considered as a general rule for many insects when they are at rest so they may exchange gases with a minimum of water loss, respiratory water loss actually contributes very little to the overall insect water budget. Data compiled from many species from four orders indicate that respiratory water loss is usually much less than 20% of the total water loss. In German cockroaches, respiratory water loss constitutes only about 3% to 4%

of total water loss. Because respiratory water loss is controlled so effectively even in insects with continuous gas exchange, the pattern of gas exchange may be unrelated to any considerations of water balance. Also, insects may switch away from discontinuous respiration at high temperatures or during periods of dehydration when water conservation would be expected to be most important.

An alternative explanation for the role of discontinuous gas exchange is related to minimizing possible oxidative damage. Because the tracheal system is efficiently designed for periods of high oxygen delivery, insect tissues when at rest are exposed to high levels of oxygen that can cause oxidative damage and lead to premature cell death. Discontinuous gas exchange cycles, which only appear in resting insects, regulate this O_2 concentration and may prevent overexposure of tissues to oxygen during periods of low aerobic demand. Consistent with this hypothesis would be the shorter longevity of metabolically active insects that do not engage in discontinuous gas exchange, but no such relationship has been identified. Although not by a mechanism of discontinuous gas exchange, some termites maintain an internal hypoxia that may allow their symbiotic gut flora to thrive under the practically anaerobic conditions they require.

AQUATIC RESPIRATION

Aquatic insects evolved from terrestrial ancestors, and various adaptations have been necessary for them to return to the water. The oxygen content of water is considerably lower than in air because of the physical characteristics of gases in water. Therefore, to obtain a comparable degree of oxygen in the water, an aquatic insect must ventilate its gas exchange surface at a much higher rate than that of an air-breathing animal. However, the spiracles of terrestrial insects are too small to function in water, and their cuticles are impermeable to gas exchange. Obviously, to enable some insects to reexploit aquatic niches, it was necessary for them to evolve certain adaptations that allowed them to breathe in water.

Renewal of Air Supplies

The least modified adaptation is found in insects that have retained the open respiratory system of terrestrial insects. With the retention of a conventional open tracheal system, the challenge was to prevent its flooding with water. Once this problem was solved, the basic strategies for survival underwater included either the total reliance on atmospheric oxygen or the capacity to bring air underwater to satisfy the insect's short-term demands for oxygen.

The problem of keeping water out of the terrestrial tracheal system was addressed through the evolution of hydrofuge surfaces, which have a greater

affinity for air than for water because of the waxes produced by glands at their base. The hydrofuge hairs that surround a spiracle will cover the spiracle when they are submerged and open from surface tension when the insect surfaces. Several metapneustic dipteran larvae, such as mosquitoes, have spiracles at the end of an abdominal siphon that allow the larvae to feed at a lower level in the water while they simultaneously respire at the surface. The hydrofuge hairs cover the spiracular opening when the insect submerges (Figure 9.17).

Another modification is the ability to capture a small air store that opens into the spiracle and allows the insect to remain active underwater for short periods. Surfacing is still necessary to replenish the bubble, but the air store extends the time it can remain submerged. For example, dytiscid beetles carry air that is held in place by hydrofuge surfaces beneath the elytra. The air store also provides the insect with buoyancy as it swims.

Several insects have retained their terrestrial tracheal system and spiracles while evolving the means to tap into underwater plants to obtain oxygen while submerged. Thus, all stages of the coleopteran *Donacia* can live at the bottom of bodies of water and, by using a sharp posterior siphon, can penetrate the roots and extract the oxygen from them. Similarly, larval mosquitoes in the genus *Mansonia* are able to remain underwater by tapping the stems of plants with their sharply pointed spiracle.

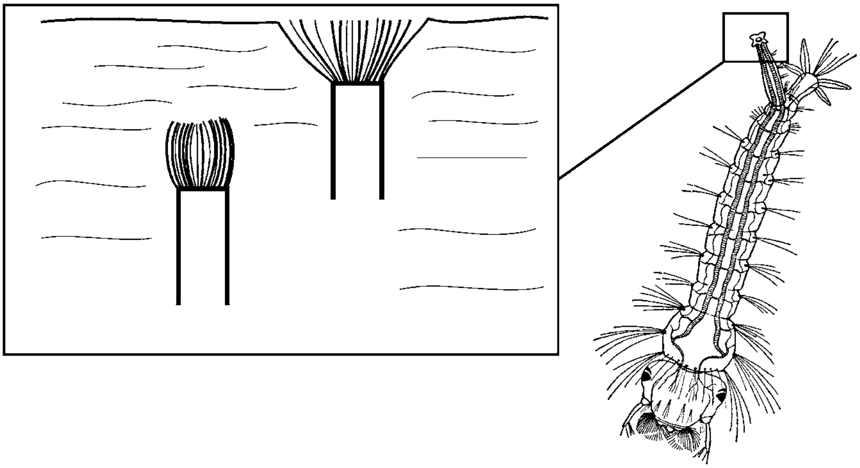

FIGURE 9.17. Hydrofuge hairs on the tip of the siphon tube of an aquatic insect. When underwater, the hairs remain over the spiracular opening and prevent water from entering. At the surface, the hairs open up and allow air to enter.

Cutaneous Respiration

Another way to prevent water from entering the tracheal system is to completely close off the spiracles and respire through the cuticle. Larvae of the aquatic dipteran, *Chironomus,* have a thin cuticle that allows oxygen to diffuse into the well-developed tracheal system beneath it. In another dipteran, *Atrichopogon*, the tracheae are associated with the cuticle in eight specific areas through which respiration takes place.

Tracheal Gills

Another step toward respiration through the cuticle is the development of tracheal gills that are outgrowths of the body wall covered by relatively thin cuticles with rich supplies of tracheae. The abdominal gills of ephemeropterans are platelike outgrowths that undulate continuously to circulate oxygenated water over their surfaces as the insect swims (Figure 9.18). Zygopteran odonate larvae have three caudal gills that are similarly configured to take up oxygen, and their undulations also serve as rudders to aid in swimming.

Tracheal gills are present within the modified hindgut of dragonfly larvae, creating a **brachial chamber** that extracts oxygen from the water contained there (Figure 9.19). The wall of the chamber is lined with circular and

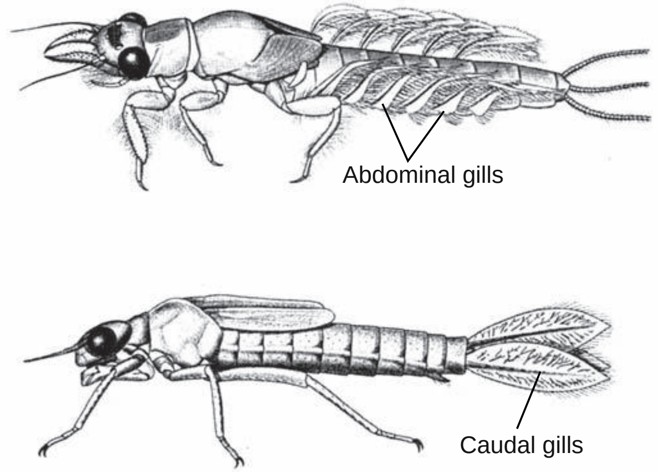

FIGURE 9.18. Abdominal gills of the mayfly. From McCafferty (1981). Reprinted with permission.

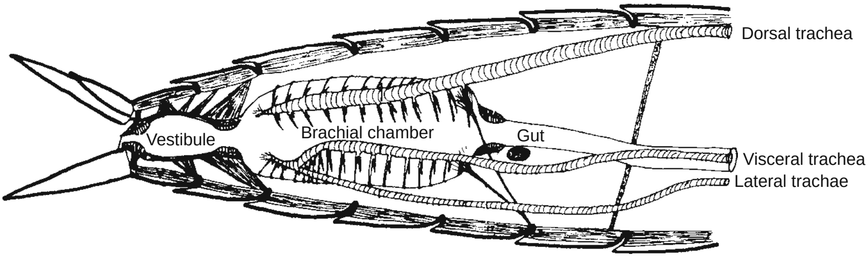

FIGURE 9.19. A longitudinal section of the abdomen of the dragonfly larva showing the brachial chamber. Reprinted from Hughes, G.M., and P.J. Mill. 1966. J. Exp. Biol. 44: 317–333. With kind permission from the Company of Biologists, Ltd.

longitudinal muscles that change its volume, causing oxygenated water to be alternately ventilated and ejected. Ventilatory contractions of the muscles of the hindgut cause water to be ejected from the anus, and dilator muscles draw fresh water into the brachial chamber. The walls are richly supplied with tracheoles that take up oxygen from the water. The rapid ejection of fluid from the anus can jet-propel the insect through the water. Approximately 85% of the water is regularly renewed. Digested food passing through the hindgut is enclosed in a peritrophic membrane and does not foul the brachial chamber with wastes.

Plastron Respiration

When the density of hydrofuge hairs on the cuticle is high, the bubble of air that the insect captures when surfacing is held tightly enough when it is underwater so that it may serve as a physical gill to extract oxygen from the water. It may be held in place between the elytron and the body wall. Initially, the bubble consists of gases in the same proportion as in the atmosphere, mainly nitrogen and oxygen (Figure 9.20). As the oxygen diffuses from the bubble into the tracheal system, the oxygen tension in the bubble decreases, causing oxygen to diffuse into the gill from the water. At the same time, the decline in oxygen tension alters the nitrogen tension in the gill, causing this gas to slowly diffuse out of the bubble until the bubble itself declines in volume and can no longer act as a gill. At that point, the insect must surface and replenish the atmospheric bubble, but the gill can provide up to 13 times the quantity of oxygen initially present. This system is also known as a **compressible gas gill** because the volume of the gill collapses as it is used. The compressible gas gill extends the period an insect can remain underwater, but its life is finite.

In insects with a permanent or **incompressible gas gill**, the bubble is held tightly by a dense array of 10^6 to 10^8 hydrofuge hairs per cm^2. Even when the concentration of oxygen in the bubble is reduced, the volume of the gill remains

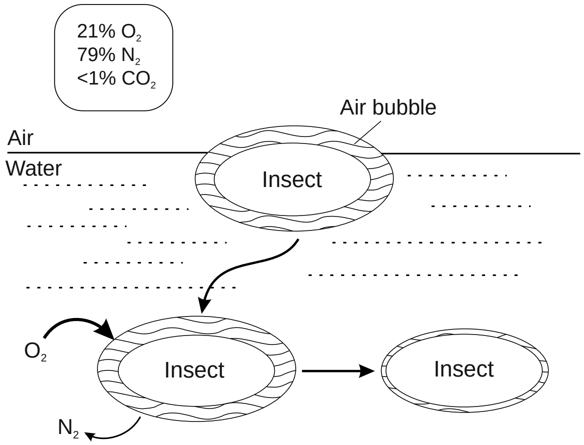

FIGURE 9.20. The mechanism of plastron respiration. When the insect surfaces, a bubble of air that is held in place by hydrofuge hairs surrounds it. When the insect submerges, the bubble remains in place. As the insect uses oxygen in the bubble to respire, the oxygen also diffuses from the water and replenishes what is lost. In insects with a compressible gill, the nitrogen eventually diffuses into the water and the insect must resurface. In insects with an incompressible gill, the hydrofuge hairs are more dense and hold the nitrogen bubble more firmly in place, allowing the plastron to operate for long periods.

the same size because of the efficient retention by the hairs. Because it remains in place over a long period, insects with incompressible gas gills can stay underwater for months without surfacing. The only requirement is that the insect remains in well-oxygenated water or the plastron will work in reverse to pass oxygen from the bubble to the water.

As described in Chapter 3, insect eggs form a relatively closed system, with the female parent packaging all the materials embryonic cells need for growth and differentiation during embryogenesis except for oxygen. The cells in the developing embryo must exchange gases, obtaining oxygen without the concurrent loss of water. The structures of the wax layer and vitelline membrane are present as a trade-off between oxygen entry and water exit. The mechanism of gas exchange in the egg is similar to cutaneous respiration in postembryonic stages, with gases able to pass through the intricate meshwork of the chorion via the numerous spaces in the chorionic framework (Figure 3.3). Insect eggs are frequently glued to substrates and thus inundated with water from rain and even dew. Given that these periods of inundation may represent a significant proportion of their embryonic stages, even these terrestrial eggs must be equipped with aquatic adaptations. The chorionic meshwork, when filled with air, may also serve as a plastron that operates when the egg is submerged for these brief periods. Some insect eggs have additional chorionic horns that bear plastrons.

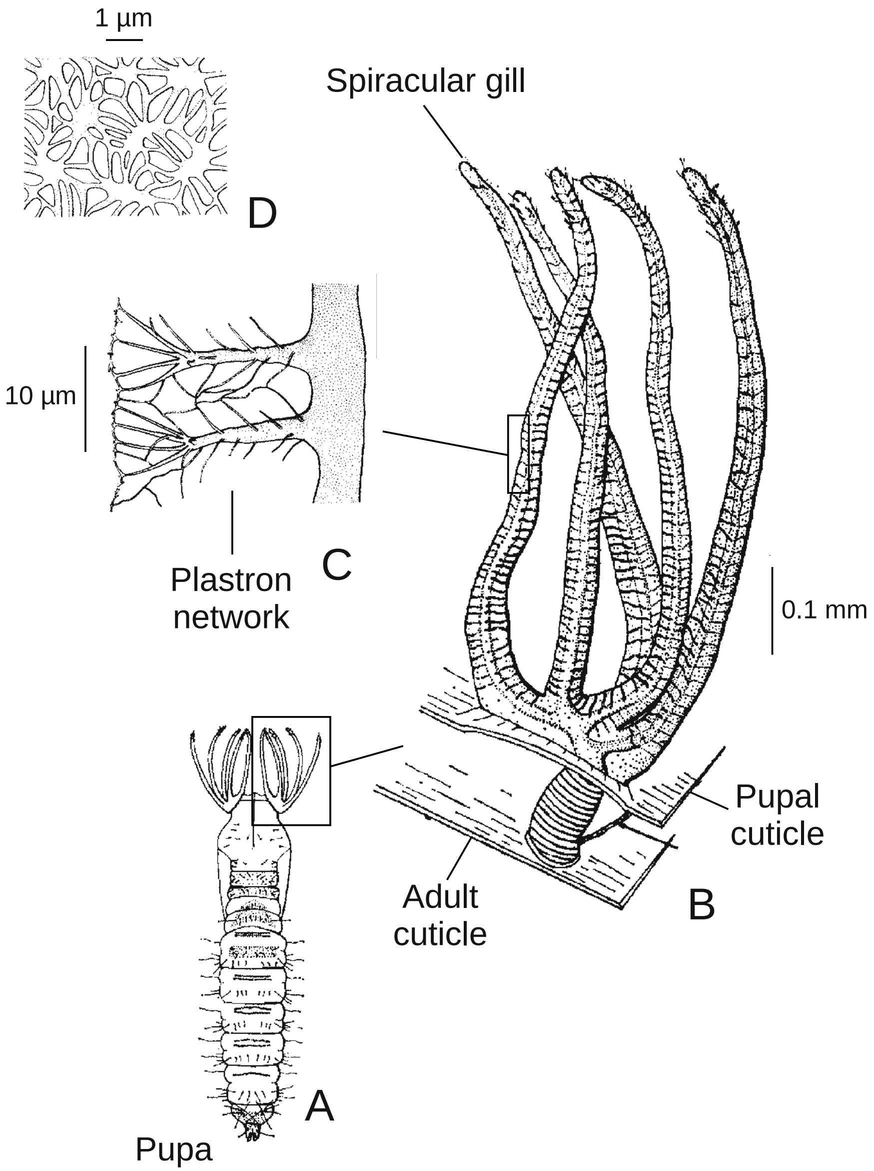

FIGURE 9.21. Spiracular gills of the black fly pupa. A. Their location on the thorax. B. The position of pupal cuticle relative to adult cuticle. C. The external plastron networks. D. The meshwork on the surface. From Hinton (1965). Reprinted with permission.

Spiracular Gills

Spiracular gills are found in insects that inhabit running water that is highly oxygenated but also subject to periodical drying (Figure 9.21). They are present in the pupal stages of several dipterans and coleopterans that inhabit intertidal environments. The gills are rigid outgrowths of the spiracle or of the body wall that are able to resist collapse from the hydrostatic pressures that may be present underwater. Most spiracular gills are covered with a plastron that is connected to the tracheal system with aeropyles, providing a large surface area for oxygen transfer to occur by diffusion when these are immersed in water. The advantage of spiracular gills is their enormous surface area for this extraction of oxygen from water while also allowing for terrestrial respiration when out of water without engendering any more water loss than would a conventional spiracle under dry conditions. When dry, the plastron allows the direct uptake of oxygen from areas closest to the spiracle with the distal remainder of the structure largely nonfunctional. Oxygen can thus be taken up without reducing the permeability of the cuticle and involving a loss of water. Compared to the more water-permeable tracheal gills, spiracular gills also offer the advantage of fewer osmoregulatory adaptations because the gills are not permeable to water and mechanisms to deal with water uptake are not as important.

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CHAPTER 10

Locomotor Systems

Anyone who has tried to catch a fly or swat a wasp will attest to the impressive accuracy and speed at which insects can maneuver. The investment made in the muscles that power this movement can be substantial; flight muscles alone can make up as much as 65% of the total body mass of some insects. At the same time, the muscular apparatus is miniscule compared to that of vertebrates. One of the smallest of all flying animals is a chalcid wasp with a wingspan of 1.4 mm and a total mass of 0.025 mg, yet it contains all the muscles and control mechanisms that enable it to fly with uncanny precision. The efficient movement of insects on land, in the air, and in water is a major factor in their domination of terrestrial ecosystems.

In addition to their maneuverability, insects can lift many times their own weight and jump many times their body lengths. The small size of insects and their apparent strength might at first suggest that their muscles are somehow different from those of vertebrates. However, there is a surprising similarity between the muscles of the two groups; the organization and the basic structure of the muscle fibers in insects are not appreciably different from that of vertebrates, except that in insects the muscles and muscle fibers are smaller and there are fewer muscle fibers in each muscle. Although the total strength of an insect is obviously less, the absolute power of insect muscle, defined by the load it can

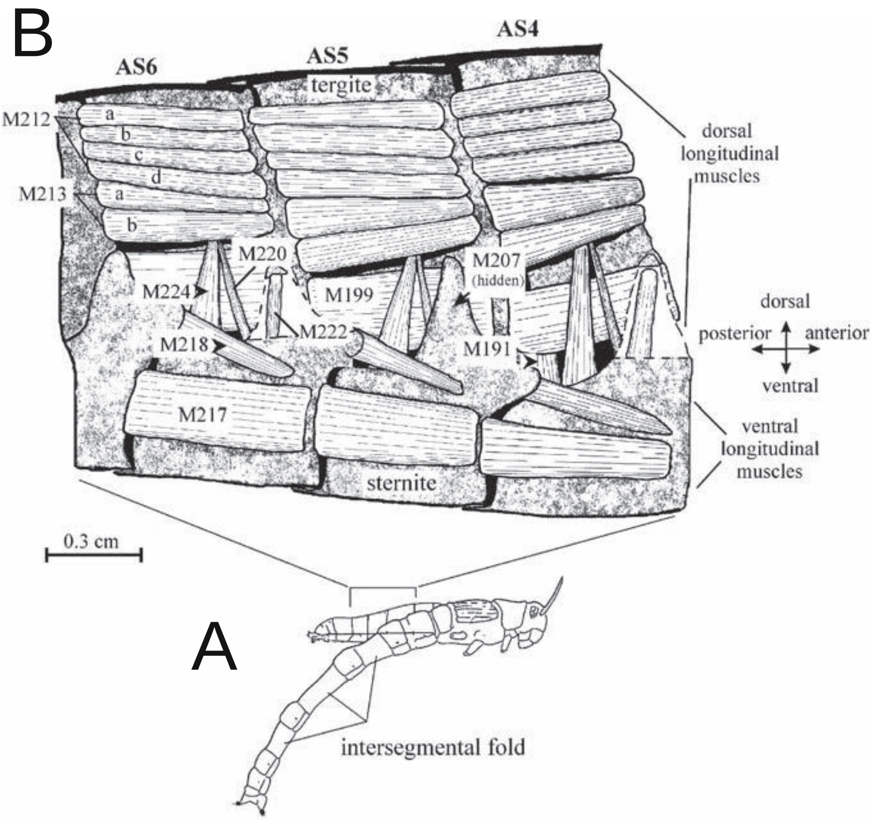

FIGURE 10.1. A. The location in the abdominal segments of the muscles involved in locust oviposition. B. Detailed view of the muscles present. Abdominal segments 4 to 6 are shown (AS4 to AS6). From Rose et al. (2000). Reprinted with permission.

raise per cross-sectional area, does not differ significantly from that of vertebrates. Their size belies their complexity; the number of individual muscles in some insect species exceeds that found in humans. Just consider the many muscles that are involved in locust flight and oviposition (Figures 10.1 and 10.2).

BASIC STRUCTURE OF INSECT MUSCLES

Muscle cells are among the most complex of all animal cells. They contain the necessary machinery for sustaining life processes and, like neurons, are capable of generating electrical signals. They have the additional distinction of being able to generate force and movement and are thus responsible for the overt

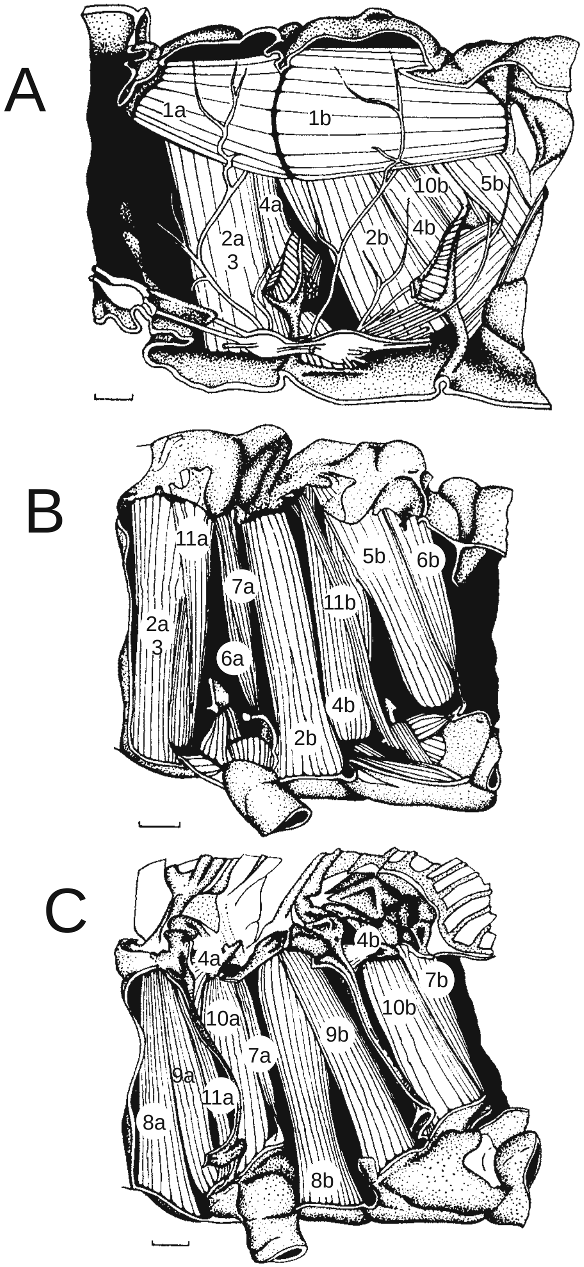

FIGURE 10.2. The thoracic flight muscles in the meso- and metathorax of a locust. A. A view from the median aspect. B. Same view, but with some of the muscles removed. C. The most lateral row of the flight muscles. Numbers are arbitrary to designate similar muscles in the two segments.

displacements of the animal that we recognize as behavior. Muscles must respond quickly and precisely to nervous signals to enable the organism to engage in complicated motions in which the timing and order of different muscle contractions are critical. Only a relatively small number of insect muscles have been studied, and none is known as well as the frog muscle that has served as the basis for much of what is known about vertebrate muscle structure. The muscles of the few insect species that have been examined show strong similarities to the structure of vertebrate muscles; many of the genes expressed in insect muscle and the resulting proteins have vertebrate homologs. Given the functional versatility of insect muscle, there is considerable variation in their structure, oxygen supply, and neural and mechanical coupling. Because the study of muscles predates modern cell theory, many of the usual terms used to describe cell structures are different when they are applied to muscle cells. For example, the cell membrane of a muscle cell is referred to as the **sarcolemma** and the endoplasmic reticulum as the **sarcoplasmic reticulum**.

All insect muscles are **striated**, owing to the regular arrangement of their components; these components produce repeating patterns of light and dark bands under the light microscope, similar to that of vertebrate skeletal muscles. The smooth muscles present in vertebrate visceral organs are absent in insects, and the insect striated muscles are capable of rapid contraction as well as the slow and considerable shortening of both of these types.

A typical skeletal muscle consists of many elongated **muscle fibers**, each of which is a single multinucleate cell (Figure 10.3). Insect visceral muscles, in contrast, tend to have muscle fibers that contain only a single nucleus. Each fiber is surrounded by an electrically excitable **sarcolemma**, an outer plasma membrane that encloses the **sarcoplasm**, or cytoplasm, of the cell. Enclosed within the sarcoplasm of muscle fibers are many smaller **myofibrils** that are arranged longitudinally and stretch the length of the fiber. The distinctive banding pattern is visible within the myofibrils because they are in turn composed of two types of overlapping **myofilaments**, consisting of the proteins **actin** or **myosin**. The thinner myofilaments consist of actin that is complexed with the regulatory peptides **tropomyosin** and **troponin**. The thicker myofilaments, with diameters of 20 nm, are composed of myosin and several other accessory proteins. A specialized endoplasmic reticulum, the **sarcoplasmic reticulum**, serves as an intracellular store for calcium and transports it from the interior of the reticulum into the sarcoplasm. Invaginations of the sarcolemma form the **transverse tubule**, or **T system**, that is closely associated with the sarcoplasmic reticulum (Figure 10.4).

The specific areas of the myofibrils where actin and myosin overlap are optically birefringent, or anisotropic, and are therefore referred to as **A bands** (Figures 10.3 and 10.5). At the center of the A band is the **H zone**, a lighter region consisting of the myosin myofilaments alone. The **M line**, absent from some muscles, represents cross-links between the thick filaments at the middle

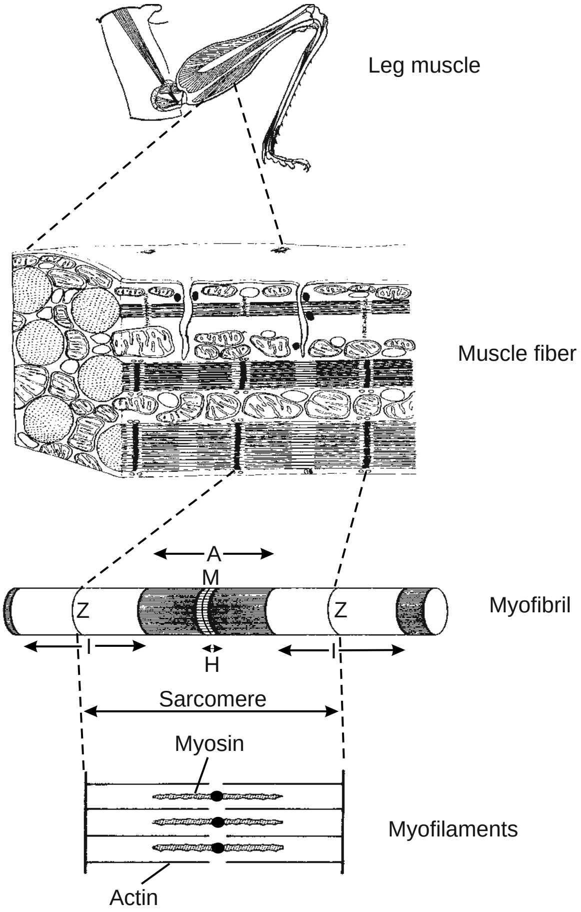

FIGURE 10.3. The structure of insect muscle. (*Top*) A leg containing several muscles that each consist of many muscle fibers. Each muscle fiber is a cell surrounded by an electrically excitable cell membrane. (*Middle*) Within the cytoplasm of muscle fibers are longitudinal arrays of myofibrils that extend its length. (*Bottom*) The banding pattern visible in the myofibrils results from the degree of overlap of actin and myosin myofilaments. The dark A band is a consequence of the overlap of actin and myosin. The H zone within the A band represents that portion of the myosin that does not overlap. The I bands are areas of actin alone, and the Z line is the actin end plate.

of the sarcomere. The regions containing only actin are isotropic and thus called **I bands**. In two-dimensional muscle cross sections, the I band is divided by a thin, dense **Z line** in which the actin filaments terminate. However, the Z line is better characterized three dimensionally as a **Z disc**. A functional contractile unit, the **sarcomere**, is defined as the area between the two Z discs. A typical myofibril may consist of thousands of sarcomeres, each about 2 μm long, that

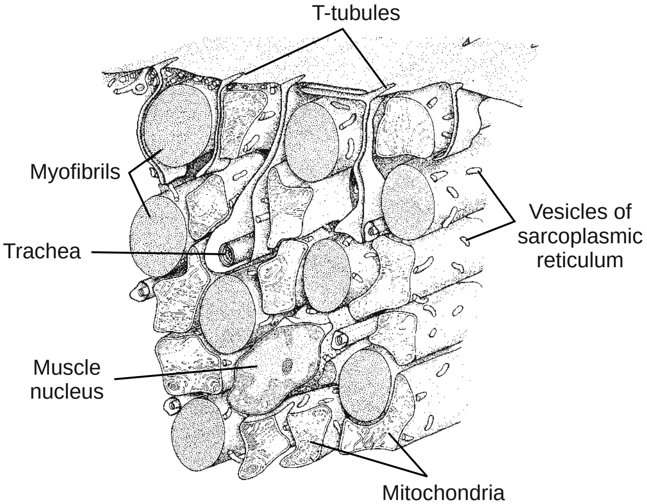

FIGURE 10.4. A cross section through a flight muscle fiber, showing the myofibrils in the cytoplasm and the invaginations of the T tubules that permit membrane depolarizations to reach inside the cell. From Pringle (1975). Reprinted with permission.

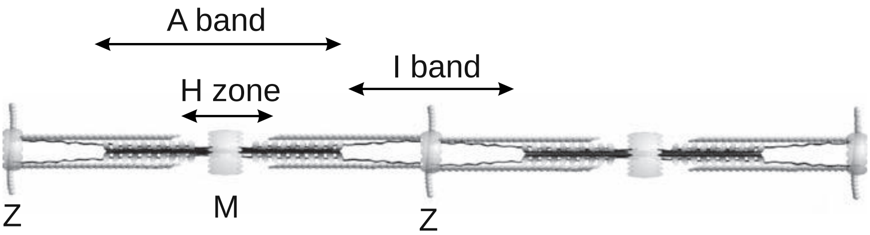

FIGURE 10.5. The morphological regions of the sarcomere with the banding pattern resulting from overlapping filaments.

are connected end to end. The sarcomeres of visceral muscles tend to be longer, on the order of 7 to 10 μm. The lining up of the Z discs accounts for the striated pattern that is visible under the light microscope.

The cross-sectional ratio of I:A bands is 2:1 in vertebrate muscle, but it varies from 2:1 to 6:1 in different insect muscles (Figures 10.6 and 10.7). In insects, a weak correlation exists between the speed of contraction and the ratio of thin to thick filaments, with a high ratio associated with a slower contraction. There is a better relationship between the length and diameter of the thick filaments, as longer thick filaments may be surrounded by more thin filaments.

Several additional proteins are associated with the insect sarcomere, many of which have vertebrate homologs. The phosphoprotein **paramyosin** is a major

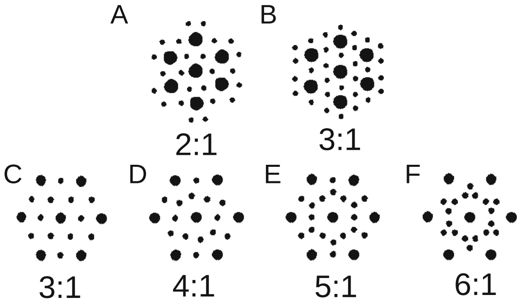

FIGURE 10.6. Cross section of actin (small circles) and myosin (large circles) from various muscles. A. 2:1 ratio from vertebrate skeletal muscles. B. and C. 3:1 ratio from insect flight muscle. D. 4:1 ratio from flight muscle E. 5:1 ratio from insect skeletal muscle. F. 6:1 ratio from insect visceral muscle. From Aidley (1985). Reprinted with permission.

Muscle type	I:A ratio
Vertebrate skeletal muscle	2:1
Lethocerus flight muscle	3:1
Neoconocephalus flight muscle	3:1
Calliphora heart muscle	4:1
Cockroach flight muscle	4:1
Butterfly flight muscle	4:1
Locust retractor muscle	5:1
Locust extensor muscle	5:1
Cockroach leg muscle	5.4:1
Cockroach intersegmental muscle	6:1
Carausius visceral muscle	6:1

FIGURE 10.7. Ratios of I:A bands found in the muscles shown in Figure 10.6. From Aidley (1985). Reprinted with permission.

structural component of the thick fibers. It forms the thick filament core with other minor proteins upon which the motor protein myosin assembles. The ratio of paramyosin to myosin within a fiber varies considerably between species and often between stages of a particular species. This ratio is 1:34 in the flight muscles of adult *Drosophila* but 1:6 in the muscles of larvae. **Titin** is a filamentous protein that extends from the Z disc to the myosin band at the center of the sarcomere (Figure 10.9). It appears to center the thick fiber, regulate its length, and provide some of the resistance to sarcomere stretch. It is associated with a kinase that may also regulate the activity of other sarcomere proteins. Another related family of 600 to 1000 kDa proteins are called **twitchins** in the worm *C. elegans* and **projectins** in crustaceans and insects. Projectin

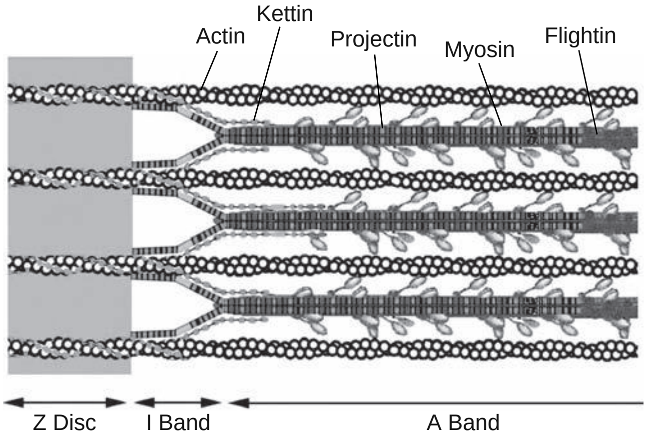

FIGURE 10.8. Proteins associated with the myosin filaments. From Bullard et al. (2005). Reprinted with permission.

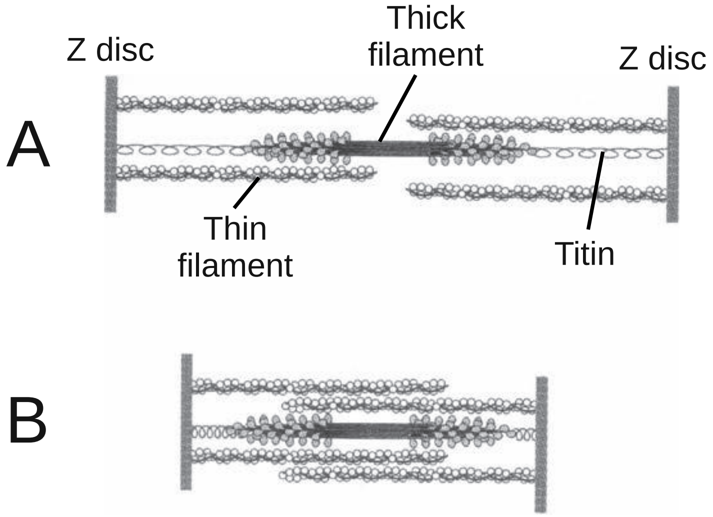

FIGURE 10.9. The location of titin stabilizing the myosin thick filaments and the sliding filament model of muscle contraction. A. Uncontracted. B. After contraction. The end plates of the sarcomere move closer together when the actin and myosin filaments slide past each other. The A band does not change in size, but both the I band and H zone decrease in size as the overlap between the myofilaments is altered.

was first identified as a 900-kDa protein that made up filaments that were "projecting" out of the Z discs of indirect flight muscles. It is located in the A band of synchronous muscles and in the I band of asynchronous types. **Flightin** is a myosin-binding protein that has so far only been found in the thick filaments of indirect flight muscles. It appears to play a role in the formation of thick

filaments and flight muscle function. A number of calcium-binding proteins have also been reported, including **calmodulin**, **troponin C**, and a **Ca^{2+}-dependent Ca^{2+} binding protein**. **Arthrin** is a 55-kDa protein associated with actin filaments that is abundant in asynchronous flight muscle and may be associated with its stretch activation. Similarly, **kettin** is a titin-like protein that contributes to the stability of the I band and its attachment to the Z disc; it provides the stiffness necessary for stretch activation.

Actin, Myosin, and Muscle Activation

A muscle is only able to contract; it can lengthen only when stretched by other antagonistic muscles. The contraction of skeletal muscles occurs when myofilaments slide past each other, shortening the length of the sarcomere as the Z discs move closer together (Figure 10.9). Although the individual myofilaments do not change in length, the overlap areas of the I band, consisting of only actin, and the H zone, consisting of only myosin, decrease in size during contraction. The unchanging distance between the Z disc and the edge of the H zone is another indication that the actin myofilaments remain the same size during contraction. The extent to which a sarcomere can contract is limited by the distance between the tips of the myosin filaments and the Z disc, as once the filaments approach the Z disc, its shortening terminates.

The shortening of the sarcomere results from the interactions between actin and myosin in the muscle fibers. The large myosin molecule consists of a number of polypeptide chains, containing at least 15 isoforms in *Drosophila* that show tissue and developmental specificity, and it is composed of light meromyosin filaments and a heavy meromyosin head. The heavy meromyosin generates the force during muscle contraction. It can be further divided into two components: a globular S1 that makes up the heads and has ATPase activity and a rod-shaped S2 region that links the heads with the filaments (Figure 10.10).

Actin is a globular protein that exists in a complex with other proteins including the tropomyosin and troponin that control the interactions between actin and myosin (Figure 10.11). The tropomyosin forms a two-stranded helical rod that runs parallel to the actin filament. The troponin complex is located at intervals along the actin filament and consists of three subunits. One of the subunits, **troponin I** (TpnI), binds to actin, **troponin T** (TpnT) to tropomyosin, and a third, **troponin C** (TpnC), to calcium. Insects have only one copy each of the TpnT and TpnI genes, compared to three copies in vertebrates. They have five to six TpnC genes, compared to two in mammals.

Reaching deeply into the sarcoplasm midway between the Z disc and the H zone where the myofilaments overlap are the invaginations of the sarcolemma called the transverse tubules. The components of this T system are closely associated with the sarcoplasmic reticulum, the network of vesicles that surrounds the

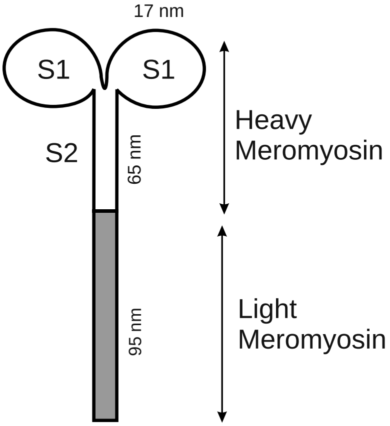

FIGURE 10.10. The structure of the myosin filament.

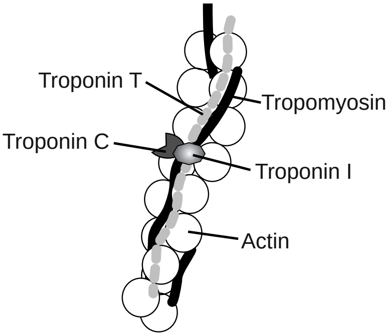

FIGURE 10.11. The composition of the actin filament, complexed with troponin and tropomyosin.

myofibrils and serves as a reservoir for calcium. An inactive muscle shows a negative resting potential of 40 to 60 mV compared to the outside. A depolarization of the sarcolemma by a nervous impulse passes to the inside of the muscle via the transverse tubules and activates the sarcoplasmic reticulum to release calcium from its internal stores in the proximity of the myofilaments. A calcium pump restores the calcium to the sarcoplasmic reticulum (Figure 10.12). When this calcium is reversibly bound by troponin, it induces a conformational change in the troponin-tropomyosin complex that exposes binding sites on the actin filament and allows the S1 heads of the myosin cross-bridges to attach to actin (Figure 10.13). The binding causes a conformational change in the myosin heads that rotates them and produces the force that moves the actin filament toward

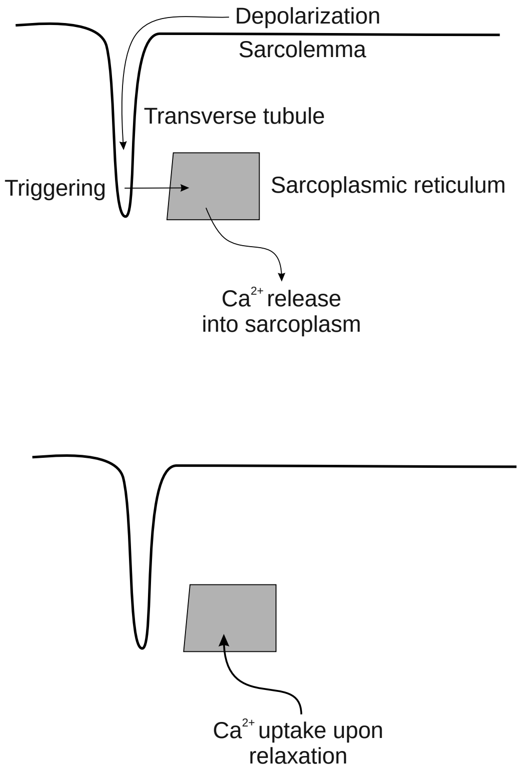

FIGURE 10.12. The depolarization of the sarcolemma reaches inside the muscle fiber via the transverse tubule, causing the sarcoplasmic reticulum to release calcium into the cytoplasm. The binding of the calcium by the actin complex triggers the contraction. The calcium is taken up by the sarcoplasmic reticulum during muscle relaxation.

the center of the sarcomere. The uptake of ATP by the myosin head causes it to detach from the actin, and the subsequent hydrolysis of ATP to ADP and inorganic phosphate repositions the myosin head for attachment to another binding site and power stroke (Figure 10.14). Thus, each myosin cross-bridge undergoes a cycle of attachment to actin, rotation, and detachment. The cross-bridge next reattaches to a new site on the actin and the cycle is repeated. The force that can be generated by a muscle depends on the number of cross-bridges that are formed.

When the central nervous system ends the excitation, calcium is withdrawn from the sarcoplasm by the sarcoplasmic reticulum, and the calcium-depleted troponin turns the muscle off by again covering the binding sites. The contraction of insect muscle is thus regulated by two events: the depolarization of the muscle by a nerve impulse and the elevated calcium concentration in the

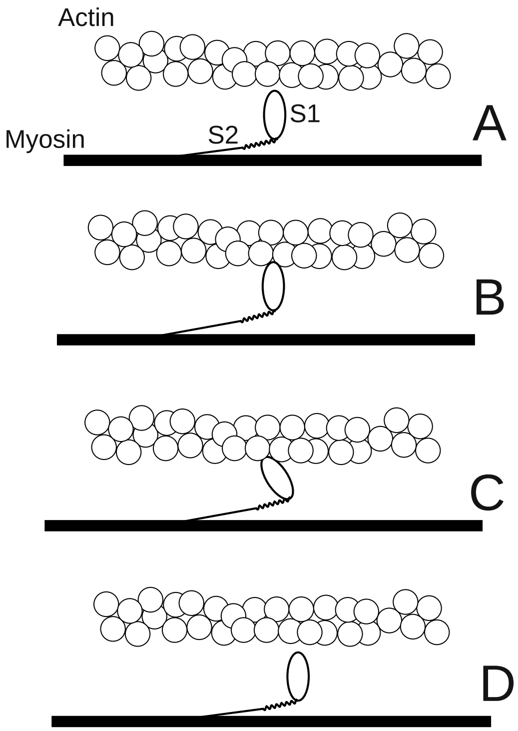

FIGURE 10.13. Mechanism of attachment and detachment of the myofilaments. A. Myosin heads are detached when the muscle is at rest. B. The S1 heads attach to actin. C. The S1 head changes in structure, causing the power stroke that pulls the filaments together. D. Binding of ATP causes the release of S1 from actin and reverses the structural change.

sarcoplasm. The nervous depolarization triggers contraction, and the elevated calcium determines when and for how long the contraction occurs.

TYPES OF INSECT MUSCLES

There are two major types of insect muscle. **Visceral muscles** surround the viscera but do not attach to the body wall. **Skeletal muscles** are anchored to the exoskeleton at either end and move parts of the skeleton relative to each other. Integumental invaginations called **tonofibrillae** are areas where the muscles attach, providing the epidermal cells with rigidity and allowing tension to be transmitted to the cuticle. Dumpy, a large, 2.5-MDa cell adhesion protein, is localized in these areas where the muscle cells attach to the cuticle through tendons. The tonofibrillae are resistant to the molting fluid that digests the

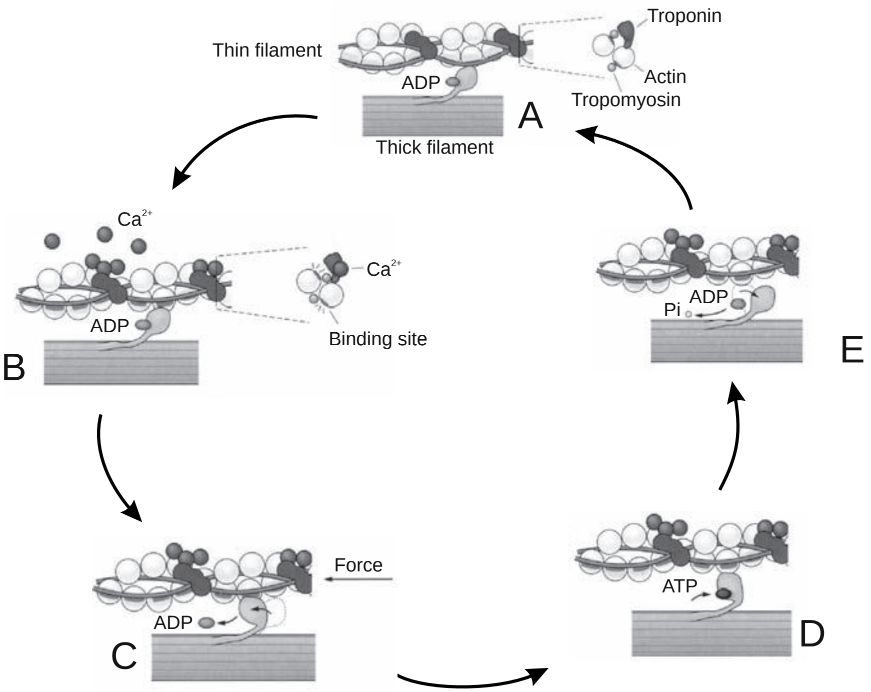

FIGURE 10.14. The steps in the cycle of muscle contraction. A. The muscle fiber at rest. Adenosine diphosphate (ADP) is bound to the myosin heads, and the troponin-tropomyosin complex has no bound calcium. B. The muscle fiber is activated when calcium is released from the sarcoplasmic reticulum and binds to the tropomyosin, causing them to expose actin binding sites. This allows the myosin heads to form cross-bridges between the thick and thin filaments. C. The attachment causes a conformational change in the myosin, and they exert forces that shorten the sarcomere by producing a greater overlap of the filaments. D. When the power stroke is completed, ATP binds to the myosin head and causes it to detach from the actin. E. The energy contributed by the ATP reconforms the myosin head for attachment to another binding site. From Kandel et al. (2000). Reprinted with permission.

endocuticle, so they remain intact during the molting process to allow the muscles to continue functioning during ecdysis. Skeletal muscles are further divided into synchronous and asynchronous muscles, based on their regulation by the nervous system.

Synchronous Muscles

Most of the skeletal muscles in insects are called **synchronous** because they contract in synchrony with nervous signals from the motor neurons that

innervate them. Each nervous signal is followed by a single contraction of the muscle, and in the case of wing muscles, with potential contraction frequencies of up to 550 Hz that commonly produce a wing-beat frequency in the range of 5 to 30 Hz. The abundant sarcoplasmic reticulum in synchronous muscle cells enables a large amount of calcium to be released when the muscles are stimulated, and the sarcomeres undergo a maximal degree of contraction. This extensive sarcoplasmic reticulum also allows the calcium to be efficiently resequestered for muscle relaxation. Synchronous muscles are found in what are considered to be the relatively primitive flight muscles of orthopterans, lepidopterans, and odonates. The sound-producing tymbal muscle in a cicada has the highest known frequency, 500 Hz, of contraction of any synchronous muscle, but the amount of sarcoplasmic reticulum that is necessary for this frequency occupies almost one third of the muscle cross section.

The contraction of most synchronous muscles is generally limited to a maximum shortening of about 50%, but there are special supercontracting and superextending muscles associated with structures that undergo an unusual degree of extension. For example, the intersegmental muscles that allow the elongation of the abdominal segments containing the ovipositor in some insects can stretch to beyond 10 times their normal length and shorten by as much as 90% (Figure 10.15). This unusual change in length is because of the penetration of the Z disc by the myofilaments during supercontraction and by the fragmentation of the Z disc into Z bodies during superextension. Supercontracting muscles have also been identified surrounding the midgut of tsetse.

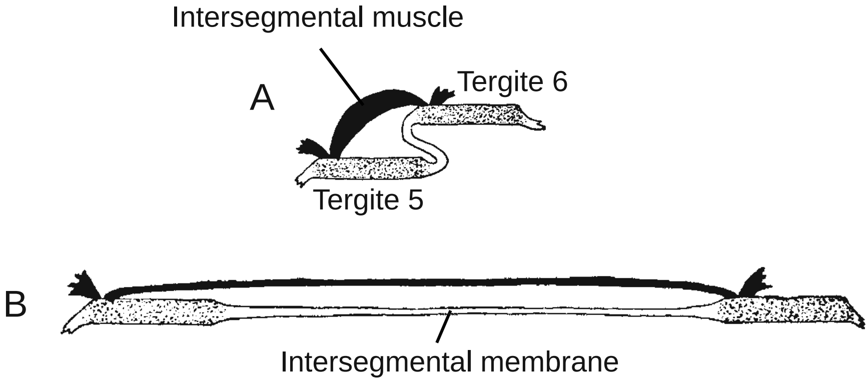

FIGURE 10.15. Intersegmental muscles allow for the supercontraction (A) and the superextension (B) and of the body segments. Reprinted from Jorgensen W.K., M.J. Rice. 1983. Superextension and supercontraction in locust ovipositor muscles. J. Insect Phys. 29: 437–448. Copyright 1983, with permission from Elsevier Science.

Asynchronous Muscles

The insects in several more advanced orders, including dipterans, coleopterans, hymenopterans, and some hemipterans, have evolved smaller wings that enable them to occupy niches unavailable to those insects with large wings. For these smaller wings to achieve the necessary aerodynamic forces to support flight, they must beat more rapidly. However, the higher wing-beat frequency that is necessary may exceed the rate at which the nervous impulses can reach the muscles. The wing-beat of synchronous muscles is limited to no more than 550 Hz because after the transmission of a nervous impulse, the neuron undergoes a brief refractory period during which time its membrane potentials are restored and no additional impulses can be transmitted. This structural design limits the rate at which a neuron can fire. If wing-beat were restricted by the speed of nervous transmission, there would be a definite restriction on the lower size limit of flying insects, because flight by the smaller insects could only be possible if their smaller wings were able to beat faster. In the ceratopogonid midge, *Forcipomyia*, the wing-beat approximates 1000 per second, which is too fast for the control of flight muscles by individual nervous impulses. The rapid contraction of its flight muscles is only possible because another type of muscle is present that does not require individual nerve impulses to stimulate its contraction.

These special muscles, found only in insects, are called either **fibrillar**, because of their relatively large myofibrils, or **asynchronous**, because they contract without the 1 : 1 synchrony with electrical events. The frequency at which nervous signals activate these muscles is considerably less than the frequency of contractions. In contrast to the one nervous impulse per contraction in synchronous muscles, asynchronous muscles generally oscillate through 5 to 25 contractions for each nervous impulse (Figure 10.16).

Asynchronous flight muscles are present in about three quarters of the known insect species and appear to have evolved independently several times from synchronous muscle, but they show the same structure wherever they are found. The sarcomeres are more compact than in synchronous muscles, with their myosin filaments closer to the Z disc that results in a reduced I band. Asynchronous flight muscles also have a reduced sarcoplasmic reticulum that has a slower rate of sarcoplasmic calcium exchange. This provides a significant energy savings by not having to pump calcium in and out of the sarcoplasmic reticulum with each contraction, so the consumption of oxygen per contraction is considerably less than for synchronous muscles. No accurate estimates of this savings have been determined, but in vertebrate striated muscle, calcium cycling represents a cost of up to 50% of the total energy expended by contraction. The reduced sarcoplasmic reticulum also allows for larger myofibrils and more mitochondria within the sarcomere. A constant level of calcium is maintained by a low frequency of nervous impulses, and self-oscillatory contractions are initiated

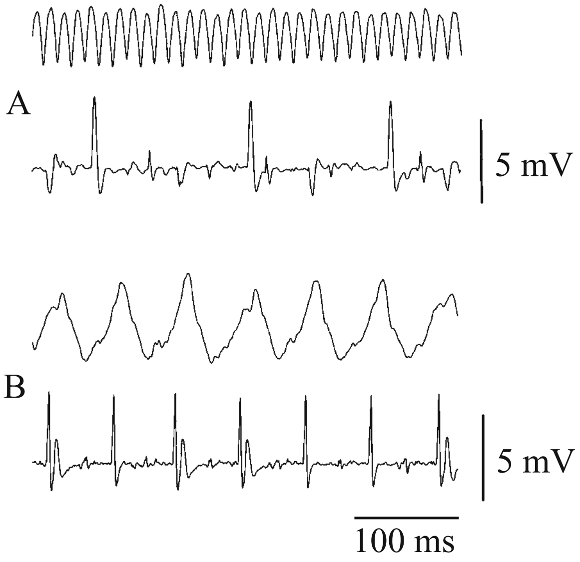

FIGURE 10.16. Traces of wing thrust (*top in each row*) and muscle action potentials (*bottom in each row*) for (A) the asynchronous flight muscles of a beetle in which occasional nervous impulses maintain a state of contractibility and (B) the synchronous flight muscles of a locust in which the nervous impulses cause each contraction. From Josephson et al. (2000). Reprinted with permission.

by the characteristics of the myofibrils. This also avoids the high cost of calcium cycling associated with synchronous muscles. Nervous excitation brings the contractile apparatus into a state of activation at which time it becomes sensitive to mechanical stretching but does not influence the frequency of contraction. The stretching may expose more myosin heads, allowing them to contact the actin molecules more readily. There is also a greater overlap of actin and myosin filaments that allows an increased number of attachment points for the lower amplitude contractions. The contraction of asynchronous muscles is on the order of only about 3% as a result of the shorter I bands than synchronous muscle contains and their increased stiffness.

In the Hymenoptera, sex determination is by haplodiploidy, in which fertilized eggs develop into diploid females and haploid males result from unfertilized eggs. In spite of their haploid development, the flight muscles of male hymenopterans are diploid. This restoration of diploidy in the muscles of the males may provide them with greater efficiency and control compared with what the haploid nuclear DNA content of muscle cells could offer.

There are several other structural differences in asynchronous muscles. Because the oscillatory contractions of asynchronous muscles are activated by their stretching, the fibers have an increased stiffness that allows them to respond to the

small changes in length. This stiffness results from the presence of several proteins mentioned earlier in this chapter. The protein **arthrin** is a form of actin that has been isolated from asynchronous flight muscles and may be involved in the regulation of muscle activation by stretch. **Projectin** projects from the Z lines into the I bands of asynchronous muscle and along with **kettin**, which binds to both actin and myosin, may contribute to the stiffness of the muscle that is necessary for stretch activation. **Flightin** is an additional novel myofibrillar protein in asynchronous muscles that appears in different isoforms as the adult *Drosophila* matures its flight capabilities after emergence. A unique accessory protein on the actin myofilaments, **troponin-H**, is found only in asynchronous muscle. Troponin-H may modulate the mechanism of muscle activation by calcium and stretching. An isoform of **troponin-C** unique to asynchronous flight muscles has a single Ca^{2+} binding site in contrast to its two binding sites in skeletal muscle.

Once activated, the stimulus for asynchronous flight muscles to contract is their stretching by antagonistic muscles. Much like the vibrations of a tuning fork, the oscillations of asynchronous flight muscle are based on their inertial load, with nervous impulses activating the muscles but not determining the frequency of their contraction. When the wings of synchronous fliers are trimmed, the wing-beat is not affected, but when the wings of asynchronous fliers are trimmed, the wing oscillations increase because the load on them has been reduced.

The storage of energy in the elasticity of the thorax is an essential property of insect asynchronous flight. Indirect flight muscles distort the thorax, and the movement of wings follows. Instability in the configuration of the thorax was once considered to be a major component of wing movement, with a so-called click mechanism the result of the positions of reduced stability in the midrange of wing movement that made extreme wing configurations less energetically expensive than others. When the wings are moved, they encounter a resistance in the thorax movements, but once this resistance is overcome, they "click" into their next position (Figure 10.17). The muscles are therefore only required to contract to a certain point to bring the wings to the area of instability and release the energy stored in the thorax in order to make the wing move fully. Once the tension is released, the antagonistic muscles that were stretched begin to contract. Rather than move the wings throughout the entire range of aerodynamic effectiveness, the muscles only have to move the wings slightly and then the energy stored in elastic proteins in the thorax is used to complete the wing movement. Energy stored in the elasticity of the thorax allows some of the kinetic energy stored in cuticular flexibility and in the flexible protein resilin to be carried over from one stroke to the next. The resistance of the indirect flight muscles to stretching may also allow the muscles themselves to store energy during their elongation. The existence of the click mechanism was originally identified in flies that were anesthetized with carbon tetrachloride, and it was

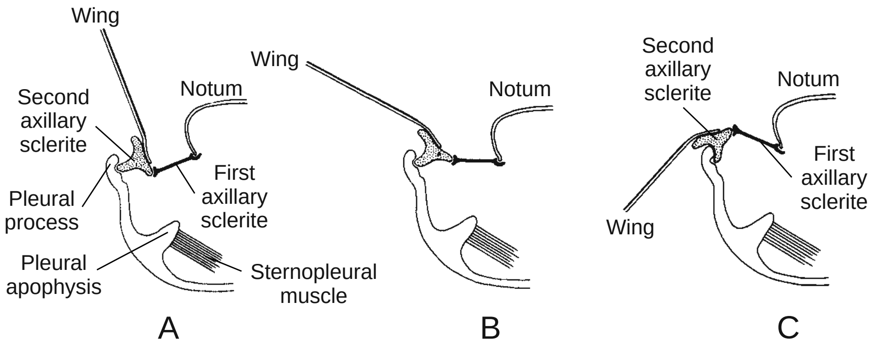

FIGURE 10.17. Operation of the click mechanism in insect flight. A. Position of stability with the wing raised. B. When wing is lowered slightly, it enters a region of position instability resulting from the thoracic configuration, causing it to rapidly click downward (C) to another position of stability.

much later suggested that this mechanism might be an experimental artifact based on an altered thoracic configuration caused by the abnormal contractions of the pleurosternal muscles.

Tracheal Supply to Muscles

The contraction of muscles requires energy and an adequate supply of oxygen. The flight muscles of insects have the highest known rates of oxygen consumption for any animal locomotor tissues. The oxygen consumption of bumblebees in flight is between 60 and 70 ml $g^{-1}h^{-1}$, compared to only 40 to 50 ml $g^{-1}h^{-1}$ for hummingbirds flying at the same speed. Compared to resting metabolic rates, flight can raise the metabolic rate in some insects as much as 50 to 200 times. In spite of the enormous energy requirements of insect flight muscles, their respiration is always aerobic. Muscles are well aerated; the area of the tracheal system in the flight muscles can be as great as 10% of their total cross-sectional area. Tracheoles are in close contact with most insect muscles to provide the necessary oxygen, but in flight muscles, they indent the muscle membrane to become functionally intracellular. They penetrate to the interior of the muscle fibers by accompanying the invaginations of the T tubules and ensuring that the oxygen is carried directly to the point of its consumption, contacting or encircling the mitochondria. At rest the tracheoles may be filled with liquid, but during flight the liquid is absorbed into the muscle cells, providing a continuous column of air through which the oxygen can diffuse. Surrounding these terminal tracheoles are abundant mitochondria that are able to utilize the oxygen carried by the tracheal system (Figure 10.18).

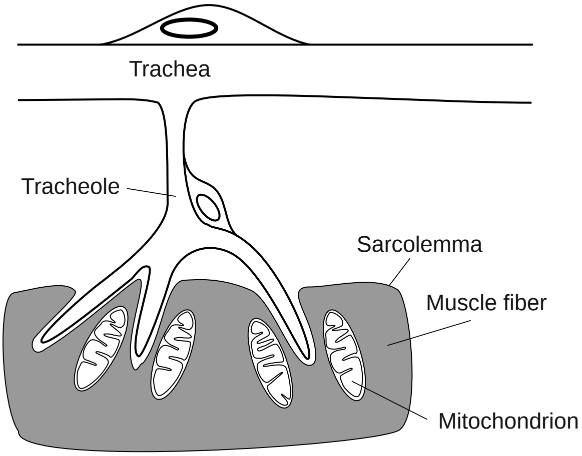

FIGURE 10.18. Penetration of the flight muscle fibers by tracheoles.

Large increases in tracheal ventilation occur during flight. Air sacs in the tracheal system change their volume as a result of their compression by wing movements, increasing the convective ventilation (Figure 9.14). Also, abdominal contractions that are synchronized with spiracular openings can produce a pumping action that drives additional air through the tracheal system.

Neural Excitation and Modulation of Muscle Contraction

A single vertebrate muscle may have hundreds of axons, but individual muscle fibers bear only single nervous innervations and are activated in an all-or-none fashion. The strength of a contraction depends on the number of total muscle fibers within the muscle that is recruited at one time, with fewer muscle fibers activated when smaller contractions are required. However, in the smaller insects, muscles often consist of only one or two fibers and the option of recruiting fewer muscle fibers for smaller muscle contractions may not exist. In these insects, graded contractions must be accomplished by some other mechanism than the recruitment of additional muscle fibers.

The motor neurons that innervate insect skeletal muscles run along the muscle fibers and repeatedly synapse at intervals. One muscle fiber may receive multiple innervations from several motor neurons, and these may consist of a combination of neurons that are designated as fast and slow (Figure 10.19). These speed designations refer to the speed of muscle contraction that the neurons produce rather than their own speed of signal transmission. For example, the large muscle

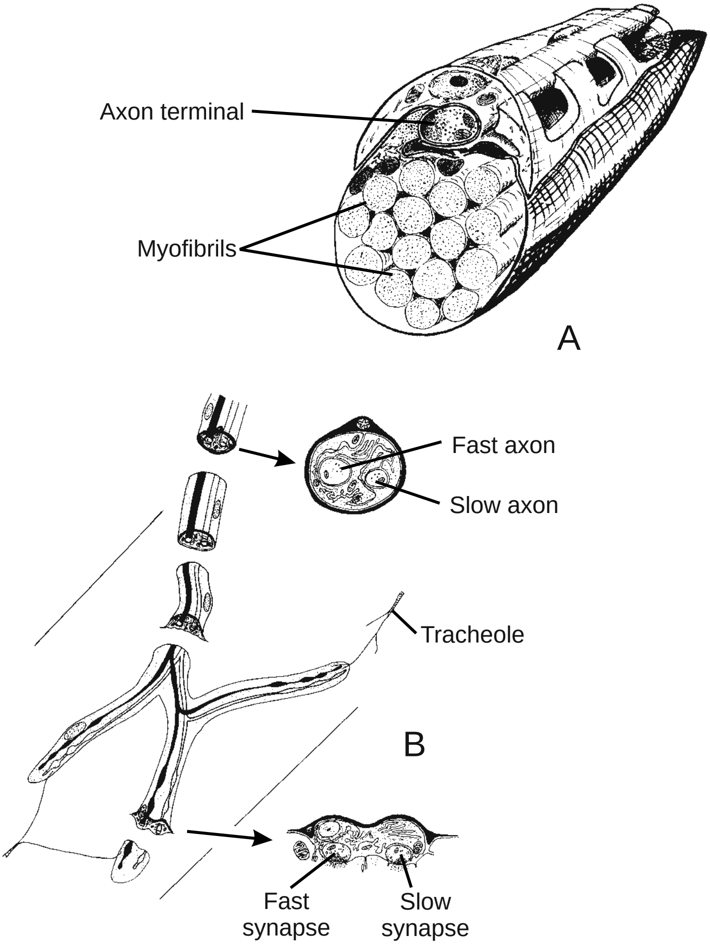

FIGURE 10.19. Populations of fast and slow axons innervating a muscle. A. The muscle cell containing the axon and myofibrils. B. Cross section of the axon showing the presence of both fast and slow axons. From Hoyle (1965). Reprinted with permission.

of the hind leg of the locust is innervated by two excitatory neurons that synapse repeatedly every 10 to 100 μm, resulting in about 100,000 synaptic contacts that control muscle contraction.

These **fast neurons** innervate all muscle fibers and cause a rapid muscle contraction (Figure 10.20). The **slow neurons** additionally innervate some skeletal muscles and cause small depolarizations and slight twitch muscle contractions. Repeated firing of the slow neurons causes a summation of depolarization effects and allows a muscle made of only a few fibers to engage in graded

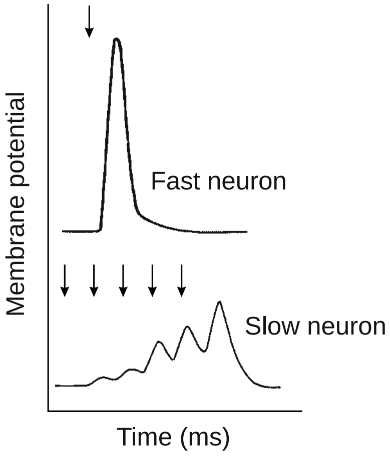

FIGURE 10.20. Changes in membrane potential after the stimulation of fast and slow neurons. In fast neurons (*top*), nervous stimulation (*arrow*) causes a sharp rise in membrane potential, leading to a rapid muscle contraction. In slow neurons (*bottom*), each nervous impulse causes a small depolarization, but their effects can be summated. This allows a muscle that consists of only a few muscle fibers to engage in a graded contraction.

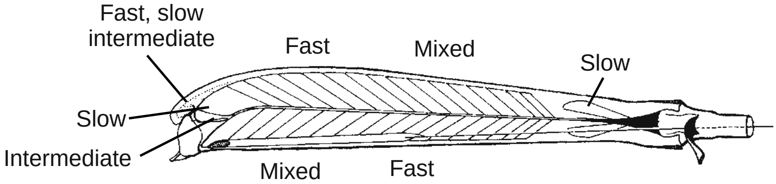

FIGURE 10.21. Populations of fast and slow neurons in an insect leg. From Delcomyn (1985). Reprinted with permission.

contractions rather than an all-or-nothing response. The jumping muscle of the locust hind leg is innervated by both fast and slow neurons, with about 30% of the muscle fibers supplied by slow neurons (Figure 10.21). These slow neurons are used for ordinary movements, whereas the fast neurons are used for leaps. In addition to these excitatory neurons, skeletal muscles may be innervated by **inhibitory neurons** that inhibit membrane depolarization when they fire. These neurons release the inhibitory neurotransmitter γ-aminobutyric acid (GABA) that acts postsynaptically to affect the muscle's response to stimulation by excitatory neurons. The multiple innervations with fast, slow, and inhibitory neurons allow the relatively simple insect nervous system to send complex neural messages to muscle groups.

The excitatory neurotransmitter at the insect neuromuscular junction is generally the amino acid L-glutamate (Figure 10.22). It is present in vesicles at the synapse and released at the muscle surface to cause a depolarization of the sarcolemma that is carried deep into the muscle by the transverse tubules. The inhibitory neurons release the neurotransmitter GABA, which causes an influx of chloride ions that hyperpolarizes the membrane and prevents the activation of the muscle fiber. Some skeletal muscles may be innervated by other neurons identified as dorsal unpaired medial (DUM) neurons that release the neurotransmitter **octopamine**, which is able to modulate the effects of other neurotransmitters. Its presence may synergize the release of L-glutamate and elevate the level of cAMP within the muscle cells. In locusts, DUM neurons release octopamine shortly after flight is initiated and have a variety of effects on the interneurons involved with flight and wing proprioreception. The octopamine also increases the power output of flight muscles and stimulates the release of adipokinetic hormone to mobilize lipid from the fat body for flight energy. Other

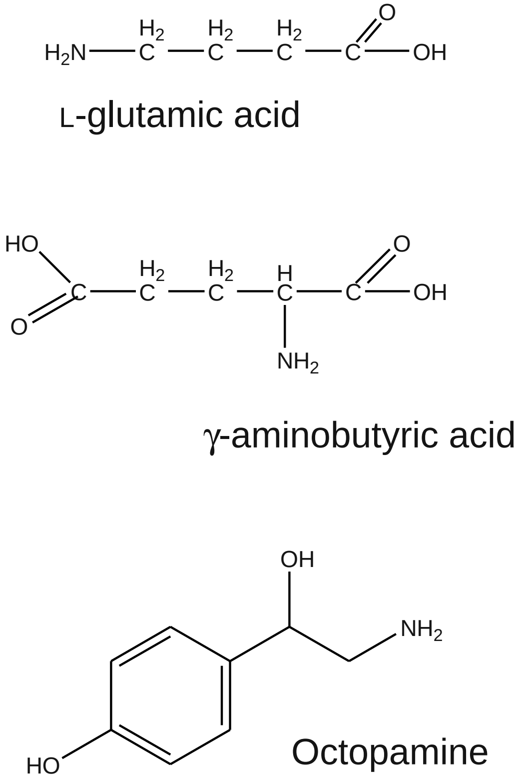

FIGURE 10.22. Neurotransmitters at the neuromuscular junction.

neurotransmitters such as 5-hydroxytryptamine, or serotonin, are neuromodulators that modify the normal response of a muscle to excitatory transmitters and increase the rates of contraction and relaxation.

Myotropic Peptides

The contraction of both skeletal and visceral muscles may be modulated by myotropic neuropeptides that produce stimulatory or inhibitory effects. The existence of these myoactive substances was first demonstrated with a bioassay that examined the contractions of a cockroach heart and later expanded to use other visceral muscles including those of the hindgut and oviduct. The first neuropeptide to be identified from an insect was **proctolin**, the pentapeptide that was originally shown to cause contractions of the longitudinal muscles of the cockroach hindgut but that has since been identified from both visceral and skeletal muscles in a wide variety of insects. It functions as a neuromodulator that works with glutamate as a cotransmitter at the neuromuscular junction. As discussed in Chapter 7, a number of **cardioacceleratory peptides** (**CAP**) control the muscles that produce the heartbeat. In newly emerged *Manduca* adults, the release of CAPs increases the heartbeat to expedite wing inflation. Small neuropeptides that have been grouped together into a family of **myokinins** have been identified from several insects. These are potent stimulators of hindgut and oviduct muscles. Some insect kinins also stimulate fluid secretion from Malpighian tubules. The insect **tachykinin-related peptides** are so named because they are related to vertebrate tachykinins. They stimulate the contraction of the visceral muscles of the foregut and hindgut, as well as the muscles of the oviduct. Tachykinin-related peptides also trigger the release of adipokinetic hormone from the corpora cardiaca of locusts. Although many of these peptides are produced by neurosecretory cells within the insect, peptides produced by the male accessory glands and transferred to females during mating may also have myotropic effects. They are able to stimulate the contractions of the oviduct muscles when they are transferred from males along with sperm.

EVOLUTION OF INSECT WINGS

Insects were the first animals on the planet to fly. The modifications that led to flight probably occurred no later than 350 million years ago and are believed to have happened only once during the course of insect evolution, with all pterygote insects descending from a common ancestor. Abiotic factors may have been different at the time and these differences may have promoted the acquisition of flight. For example, the atmosphere was believed to be significantly denser during this period, with concentrations of oxygen as high as 35% about 300

million years ago compared to the current value of 21% (Figure 10.23). The density of the air is the major determinant of force production by an airfoil, be it biological or mechanical, and the increased density may have improved the chances that flight would evolve. Accompanying increased oxygen concentrations in the atmosphere were increases in tracheal diffusion and body size. Early flying insects were enormous compared to present-day insects; the largest known insect was an odonate ancestor living during the Carboniferous with a wingspan greater than 70 cm and an estimated mass of 200 g.

Taking to the air was probably the most important evolutionary step in the history of insects, conferring significant advantages in finding food, locating mates, colonizing new habitats, and escaping predation. Two major hypotheses attempt to explain the evolution of wings from unwinged ancestors, but no transitional forms exist and there are none from the fossil record. The first hypothesis proposes that wings developed from thoracic winglets or **paranotal lobes** that might have been first used for thermoregulation, then as airfoils as they enlarged, and finally as flapping wings. The secondary wing articulation and musculature had to evolve accordingly. The small size of insects assures that they would have escaped harm from falling from any height, and the development of any new appendages would have been beneficial in gliding or in aiding their dispersal in the wind.

The second hypothesis suggests that wings evolved from winglets that originated as gills. In this second case, protowings could have first evolved in aquatic larvae and enlarged to allow improvements in underwater navigation. Once the ancestors left the water, the significance of the wings could have shifted to a second function as locomotor devices. This **articulated gill theory** has support in the behavior of some extant aquatic stoneflies that engage in surface skimming in which the wings are flapped but the insects remain in the water, a behavior intermediate between swimming and flying. The aquatic gills might have been

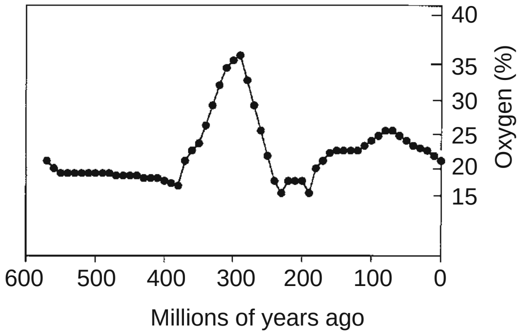

FIGURE 10.23. Atmospheric oxygen over geologic time. From Berner (1999). Reprinted with permission.

first used to "row" within the water, and they then became large enough for skimming and finally flight. The transitional use of abdominal gills that were in contact with both the air and water for rowing gave early winged insects a chance to exploit the increased concentration of oxygen in air compared to that dissolved in water. The reduced muscle power and wing movements used in skimming are sufficient to propel them through the water and explain the initial evolutionary benefits of wings before insects actually left the ground.

Molecular data provide more evidence for the articulated gill theory. The ancestral arthropod limb segment is called a **podite**. Lobes along the inner (endite) and outer (exite) margins of the podite developed in some arthropod groups. The basal segment of the limb that is attached to the pleural wall is the **coxopodite**, and its outer lobe is known as the **epipodite**. In crustaceans, the epipodites are often modified into gills (Figure 10.24). Two ancient genes that have wing-specific functions in insects are also expressed in the epipodite gills of crustaceans. The expression of the same genes in the epipodites of crustaceans and the wings of insects suggest that insect wings evolved from these epipodites. The crustaceans consist of the only group that has been largely confined to the water or moist habitats. When terrestrialization occurred in the other groups, the epipodite gill was believed to have been modified into a wing in the line leading to pterygote insects, while it was lost in the other two groups (Figure 10.25).

Because insect wings arose not from walking legs as in birds and bats but as outgrowths of the body wall, they developed as a sandwich of two epicuticular layers with hemolymph, nerves, and trachea in between (Figure 10.26). Procuticle is absent, so chitin does not appear to be present in the membranous areas of the wings. Another difference between the wings of insects and those of other flying animals is that no intrinsic muscles are associated with the insect wing. Wing movement and changes in wing shape during flight must be controlled by muscles in the thorax, with some deformations of the wing resulting from

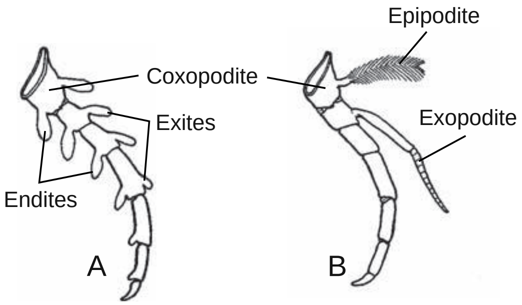

FIGURE 10.24. A. A generalized trilobite limb. B. A generalized crustacean limb. From Snodgrass (1935). Reprinted with permission.

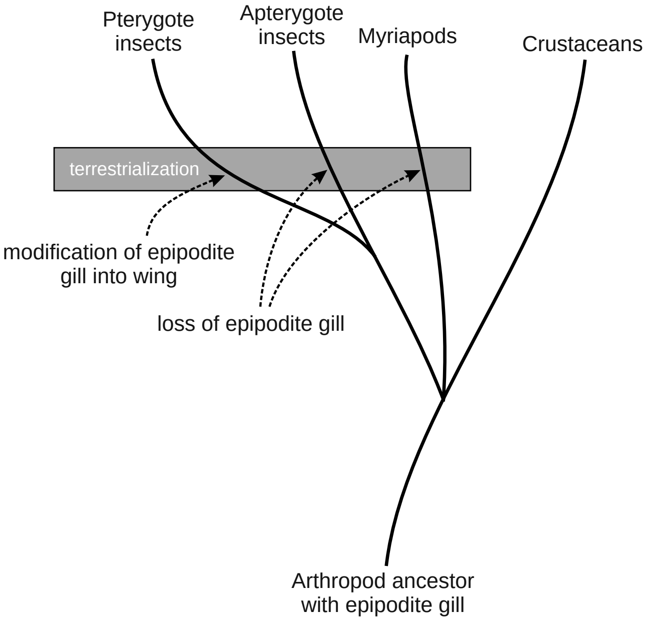

FIGURE 10.25. The possible evolution of insect wings from the epipodite gills of an arthropod ancestor. The epipodite gill remained in aquatic crustaceans, but after the terrestrialization of insects and myriapods, it evolved into the wing of pterygotes and was lost in apterygotes and myriapods. From Averof and Cohen (1997). Reprinted with permission.

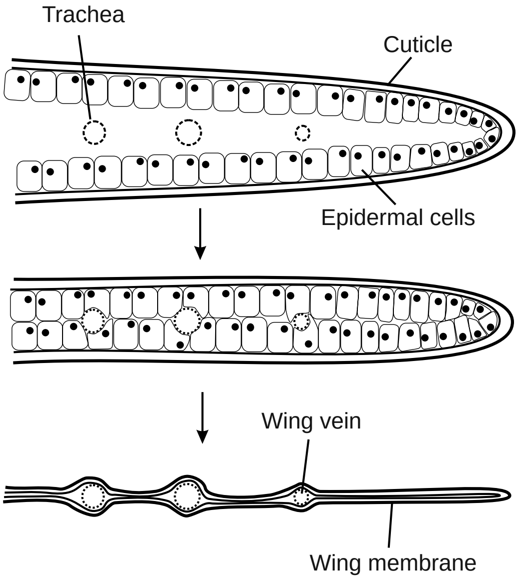

FIGURE 10.26. The development of the wing (*bottom*) from a sandwich of epidermal cells during pupal development (*top*).

the wing structure itself as it responds to the aerodynamic forces. Most of the epidermal cells in the insect wing degenerate after adult emergence, leaving a largely acellular membrane lined with veins, tracheae, and nerves. The loss of water as these cells degenerate makes the flapping movements of the wings more efficient. However, the absence of epidermal cells in the mature adult wing is also a reason that adult pterygotes no longer molt. Without functional epidermal cells, it is impossible to undergo the steps in molting and produce a new wing cuticle. Selection has favored the wing efficiency that comes with epidermal cell degeneration over the possible advantages of adult molting.

The development of flight was accompanied by an increased capacity for information processing by the insect central nervous system. The **flicker fusion frequency** is the maximum rate that individual light impulses can be resolved and is a measure of the speed of optical processing. In humans, the flicker fusion frequency is less than 20 Hz, but it can reach as high as 200 to 300 Hz in flying insects.

Basic Structure of the Insect Wing

Fully functional wings are found only in adult insects. The membranous wings are reinforced with rigid longitudinal and cross veins that may contain nerves and tracheae that are nourished by the hemolymph that flows through them. The wing hinges may contain the protein resilin that provides the necessary flexibility. In beetles that must fold their hind wings underneath the elytron, portions of the wing itself contain resilin in areas subject to repeated folding. Although most of the epidermal cells of the wing have degenerated to leave only the epicuticular sandwich, a blood supply in the wings is essential to maintain their mechanical properties and prevent brittleness. Because wings arose only once in insect evolution, the pattern of veins present in a particular insect reflects its ancestry and evolutionary relationships among other insect groups and can be used diagnostically to classify insects. More advanced insects generally have smaller wings and fewer veins. The wing may be thrown into longitudinal corrugations behind the anterior margin to provide resistance to bending, and the veins may be located on the fanlike pleats, with convex veins on the crest and convex veins in a trough (Figure 10.27). A system of wing folds allows the deformation of the wing to occur during pronation and supination and permits the wings to fold at rest. Axillary sclerites connect the wing to the thorax and permit flexion of the wing over the abdomen.

A number of surface structures may appear on the wings. Scales are borne on the wings of lepidopterans and on the hind margin of the wings of mosquitoes. Spines may be present, as on the wings of odonates. Both tactile and proprioreceptive sensilla can also be found. Little is known about the roles of these accessory structures on flight. The scales may aid the escape of lepidopterans from spider webs.

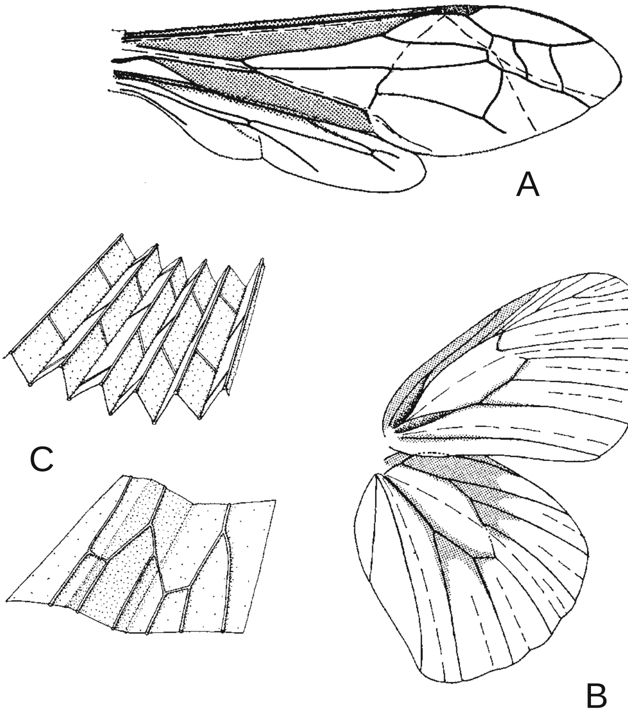

FIGURE 10.27. Corrugations and fold of the wings of hymenopterans (A) and lepidopterans (B). C. Wing fluting over weak crossveins and lines of flexion. From Wooton (1981). Reprinted with permission.

Reynolds Number, Size, and Insect Movement

Whenever an object moves through a fluid medium such as air or water, forces are generated on the object by the elements of the fluid. The relationship between the viscous frictional forces from the fluid and the inertial force of the object can be quantified and expressed as the dimensionless **Reynolds number** (**Re**). Re represents the ratio of the inertial and frictional forces. A low Re is produced by small size or low speed and indicates that frictional forces are large, whereas a high Re means that inertial forces are more important. For example, a toothpick with a low Re could be easily propelled in water but would be stopped by frictional forces from the viscosity of the water when the propulsion ceased. In contrast, a log floating in the water with a high Re would be initially difficult to move, but once it started moving, inertial forces would maintain its movement even when it was no longer pushed. The range of Reynolds numbers for living things is large, extending from approximately 10^{-6} for bacteria to 10^{7}

for animals the size of whales. Insects have relatively small Reynolds numbers ranging between 10^0 and 10^4, indicating they are especially subject to the frictional forces in the water and air in which they must move. The Reynolds number for a hummingbird is 15,000, but is only 200 for *Drosophila*, and it is as low as 15 for a tiny chalcid wasp. Insects living at a low Reynolds number experience the world in a very different way than do larger vertebrates.

The effect of the viscous medium on such small animals is much like our swimming in molasses. Among the implications of living at a low Reynolds number is that when fictional forces predominate, any movement is energetically expensive. Animals that are the size of insects encounter relatively huge energy costs to move about, and the aerodynamic strategies differ from those used by larger animals that fly. Metabolic rates during flight in some insects are as much as 100 times greater than those at rest, and the thoracic muscles of insects in flight exhibit the highest metabolic rates of any tissue. As mentioned previously, the metabolic rate of a bumblebee in flight is about 65 ml of oxygen per gram of body mass per hour, compared to about 45 ml/g/h for a hummingbird flying at the same speed. There is also a considerable inefficiency in the conversion of chemical energy to mechanical energy. As much as 80% or more of the energy used for insect flight is dissipated as heat.

The one advantage of small size is the relative strength it confers, but the apparent strength of insects lies not in any differences in their muscles but in the relationship between the increase in body volume and muscle power that accompanies increases in body size. The power of a muscle varies with its cross-sectional area, which is a function of the square of its dimensions. The volume, and accompanying weight of an animal that is powered by the larger muscle, increases with the cube of its dimensions. As animals get larger, their relative strength diminishes because their weight increases at a faster rate, a cubic function, than their muscular strength, a square function. Smaller insects benefit from their reduced size and lightweight exoskeleton.

MUSCLES INVOLVED IN WING MOVEMENTS

The flight muscles are usually the best developed of the muscles in the insect body and occupy most of the space in the thorax. Total flight muscle mass must be greater than 12% of body mass to support the weight of an insect in flight. The flight muscles of many insects undergo a maturation during the adult stage. Some dragonflies may more than double their flight muscle size as the adults age. The growth and maturation of the longitudinal skeletal muscles of the locust ovipositor are JH dependent, allowing these muscles to contribute to oviposition efficiency once the insect becomes reproductively competent. Flight muscles may also degenerate after adult emergence in order to reallocate nutrients for reproduction or diapause. This programmed cell death is triggered by environmental

signals that stimulate increases in JH, which directly affects the muscles. Flight muscle histolysis can also be triggered when the adults of some crickets shed their wings. Cricket species exhibit a wing dimorphism that determines their dispersal polymorphism. The developmental switch that commits an individual cricket to a particular morph occurs during one of several critical developmental windows. Switching to a nondispersal morph diverts the investment in wing muscles to reproduction.

There are three general categories of muscles that power insect flight: **direct**, **indirect**, and **accessory**. Direct flight muscles, consisting of the **basalar** and **subalar** muscles, insert directly at the base of the wing and provide the power for the downstroke in more primitive insects and also affect wing pronation and supination (Figure 10.28). Another direct muscle, the **third axillary** muscle, inserts on the third axillary sclerite. It affects wing supination and is also responsible for wing flexion against the body wall when the wings are at rest.

In contrast, the indirect flight muscles indirectly move the wings not by moving them directly but by changing the conformation of the thorax. These muscles include the **dorsoventral** group that extends from the tergum to the sternum. Because of the structural relationships among the sclerites that make up the thorax, in all insects, when the muscles pull the tergum down they indirectly cause the wings to raise and produce the upstroke (Figure 10.29). Another indirect group, the **dorsal longitudinal** muscles, attach longitudinally between the two phragmata of each wing-bearing segment. When these contract, they

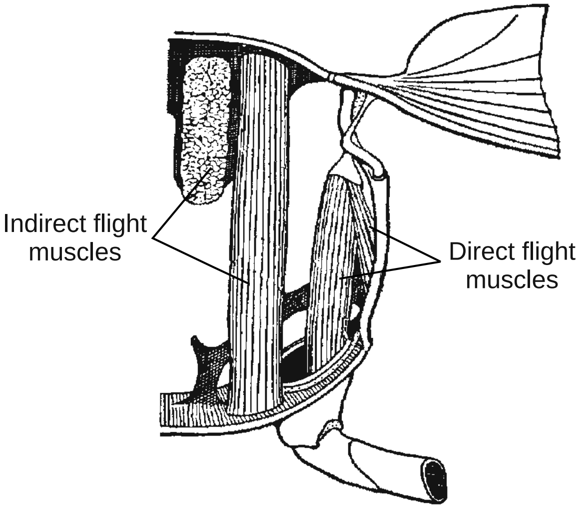

FIGURE 10.28. Cross section of a generalized insect thorax. The indirect flight muscles change the shape of the thoracic box. The direct flight muscles connect directly to the wing insertion. From Snodgrass (1935). Reprinted with permission.

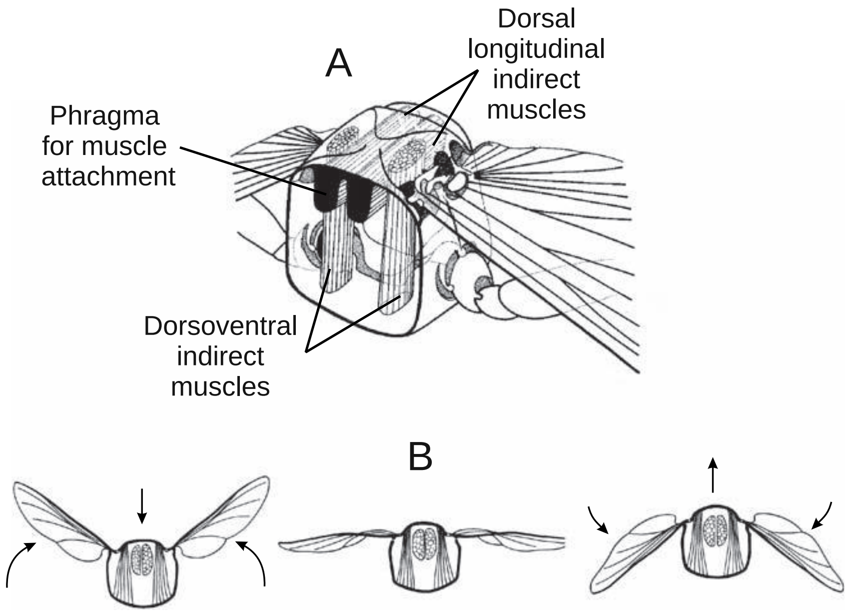

FIGURE 10.29. The mechanism of wing movement. A. The indirect dorsal longitudinal muscles attach to the phragmata at either end of the segment, causing it to shorten and move the wings downward. The indirect dorsoventral muscles move the tergum downward with contraction, causing the wings to move upward. B. Wing movements as a result of changes in thoracic conformation. From Brodsky (1994). Reprinted with permission.

shorten the segment and cause the tergum to elevate. In more advanced insects, this deformation of the notum by the dorsal longitudinal muscles produces the power stroke by depressing the wing. These muscles are reduced in more primitive insects that instead use the direct muscles for the downstroke (Figure 10.30).

There are also accessory muscles that insert into the thorax and influence its mechanical conformation. For example, the **pleurosternal** and **pleurotergal** muscles modulate the power output and the nature of the wing-beat by changing the orientation of the thoracic plates and the resonance of the thorax (Figure 10.31).

Wing Movements during Flight

Identifying the manner in which insect wings provide the necessary lift for flight has been extremely difficult. Their small size and rapid wing-beat make measurements problematic, and collecting data from tethered insects may often yield data

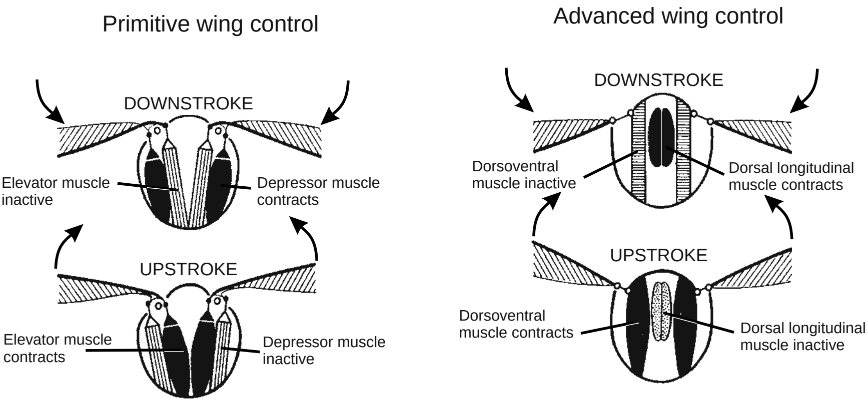

FIGURE 10.30. The differences between the more primitive wing control by direct flight muscles (*left*) and the more advanced wing control by indirect flight muscles (*right*). From Nachtigall (1989). Reprinted with permission.

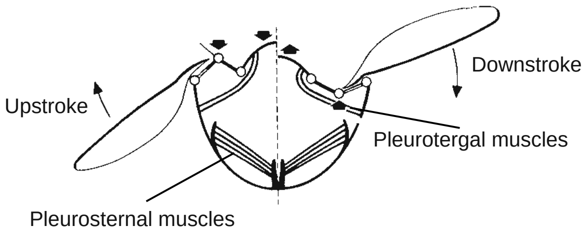

FIGURE 10.31. The pleurosternal and pleurotergal muscles are accessory muscles that change the shape of the thorax and modulate the output of the indirect flight muscles. From Nachtigall (1989). Reprinted with permission.

based on unnatural movements. Alternatively, recording from an untethered insect in flight is almost impossible. Some principles have been gleaned from scale models, but these may lack the elastic wing response and are not capable of becoming airborne.

Unlike birds that can glide for sustained periods, most insects must constantly move their wings to generate the forces that will keep them in the air. However, simply flapping the wings alone would not create the necessary downward forces, as the downstrokes would be aerodynamically canceled out by the equivalent upstrokes. Deforming the wings during the flapping is therefore necessary to generate more upward forces than downward forces. There are several ways that insects can deform their wings, including twisting them to change the angle of attack during the stroke and altering the curve of the wing from its leading edge to its tip or its camber (Figure 10.32). Insects with two pairs of wings may vary the overlap between them during flight to change the total surface area during flapping. Flight is controlled both by the frequency of wing-beat and by the

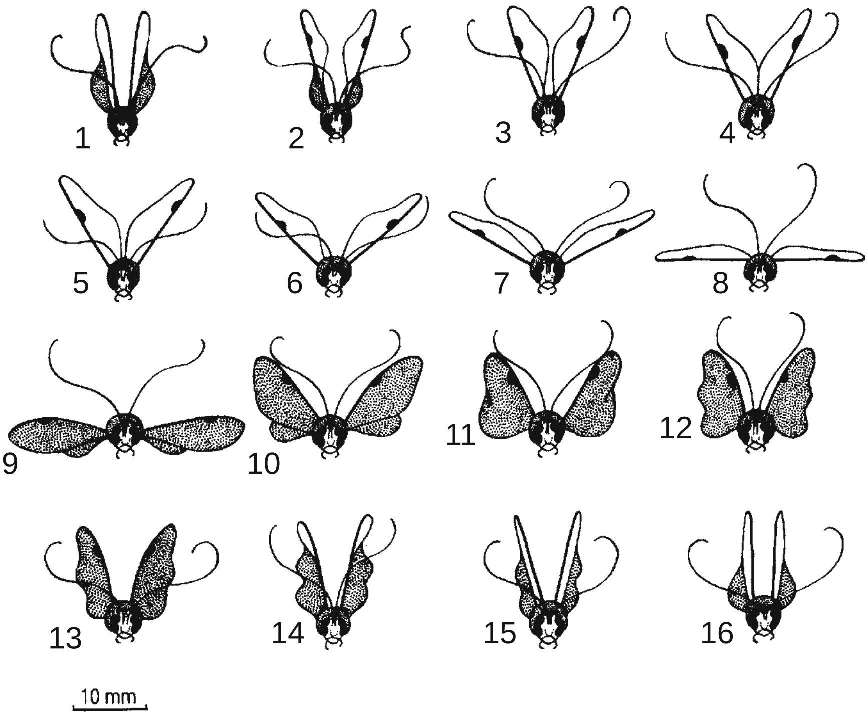

FIGURE 10.32. Successive wing movements of an ichneumonid wasp during flight. The shaded areas represent the undersurface of the wings. From Brodsky (1994). Reprinted with permission.

mechanical properties of the thorax, whose rigidity can be modified by accessory muscles.

Flight muscles not only power the wings but also modify their control surfaces. The power movements consist of an alternating upstroke and downstroke that are dependent on the alternating contraction of the elevator and depressor muscles. Separating these movements are the pronation and supination of the wings about their longitudinal axes as they reverse their up and down directions. The wing is pronated during the downstroke so that the leading edge faces downward and is supinated during the upstroke so that the leading edge is up.

In aircraft, birds, and insects, a wing produces lift only when a pressure gradient is created between the upper and lower surfaces of the wing as low pressure air flows over the upper surface. In *Drosophila*, the rotation of the wing produces a circulation of air in the opposite rotational direction and a wake capture that uses the vortex from the previous stroke to generate airflow around the wing. By capturing this lingering vortex wake, a strong force-generating airflow is created that supports flight. The air is forced backward and downward, moving the insect forward, generating thrust, and upward, generating lift.

This mechanism of wing movement used by some small insects and also butterflies is called the **clap and fling**. The clap involves the raising of the wings so the tips touch dorsally with the leading edges touching before the trailing edges, forming a vertical plate (Fig. 10.33). The wings are then quickly flung open so the gap between them is filled with entering air, and as they separate and twist, each carries a vortex of air that was formed during the fling. The bound vortex is added to the flow pattern of air that would be created by the simple wing movements. For insects that fly at a small Reynolds number, the viscosity of the air is important as air circulation becomes difficult and vortices created by the wings do not remain for long.

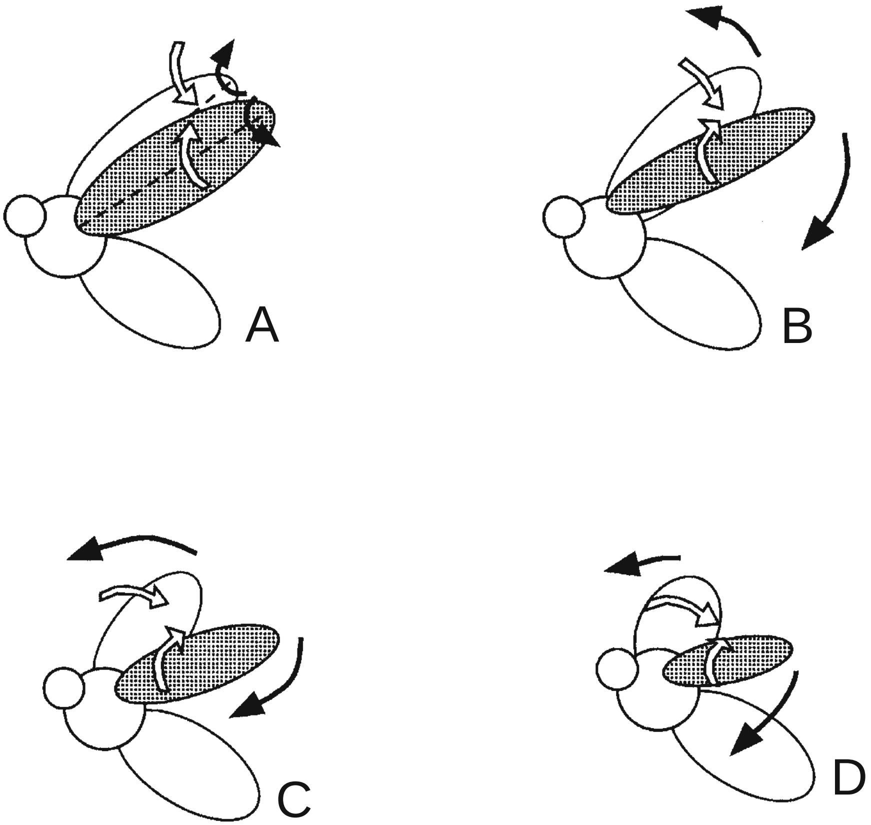

FIGURE 10.33. The clap and fling mechanism of insect flight. A. The wings clap together at the end of their upstroke, allowing air to fill in the gap between wings as they initiate pronation. B. As the wings initiate the downstroke, air continues to flow into the gap. C. As the wings further separate, the air circulation increases. D. Air circulation is fully established as the downstroke is completed. From Dudley (2000). Reprinted with permission.

Larger insects may rely on the phenomenon of **delayed stall** in which the leading edge of the wing is held at a sharp angle and creates a vortex that remains closely bound to it as well as a low pressure area that generates lift. An airplane wing performing the same maneuvers would lose lift and cause the plane to stall, but the leading edge vortices formed by the insect wing generate extra lift. Two additional mechanisms come into play between strokes when the wings are rotated. **Wake capture** allows the wing to take advantage of the wake created by the previous stroke. **Rotational circulation** allows an upward lift to be generated when the wing's own rotation creates air circulation at the end of a stroke. This is similar to the mechanism that accounts for a spinning baseball's curve as it is thrown toward home plate. These three mechanisms account for a significant amount of the lift that allows larger insects to remain airborne.

Wing Coupling and Control Mechanisms

The evolutionary trend in insects has been toward a reduction in wing size and number. The primitive condition is to have two pairs of wings that are minimally coordinated and beat independently, although their close association on neighboring thoracic segments certainly influences their movements. This is not the most efficient mechanism because the hind wings must operate in the area of turbulence generated by the fore wings.

A more advanced approach to flight is the loss of one of the wing pairs or the coupling of the two wings so they serve as one functional unit. In many Coleoptera, the fore wings are rigid and serve a protective function as a sheath when the wings are closed, but they are held open during flight while the hind wings beat. In other groups, notably the Hymenoptera and Lepidoptera, the two wings are mechanically coupled by lobes or spines at the base of the wing. In some Lepidoptera, a **jugal lobe** at the base of the fore wing overlaps with the hind wing, causing them to beat together. In other Lepidoptera, a spine or **frenulum** at the base of the hind wing may engage a catch on the fore wing. Many hymenopterans have a row of hooks, the **hamuli**, along the margin of the hind wing that catch along a sclerotized fold in the fore wing (Figure 10.34).

In dipterans, the second pair of wings has been eliminated rather than coupled. These wings are modified into knoblike **halteres** that beat antiphase to the fore wings during flight (Figure 10.35). The halteres monitor torque during flight by measuring the stress in the sensilla at their bases like a gyroscope. In the blowfly, *Calliphora*, there are more than 300 of these sensilla that monitor the insect's flight. Rotation of the body during flight causes a deflection of the halteres that is detected by the sensilla. The nervous signals from these sensilla are sent directly to the neurons that control the flight muscles and alter the wing-beat accordingly to control steering (Figure 10.36). During dipteran

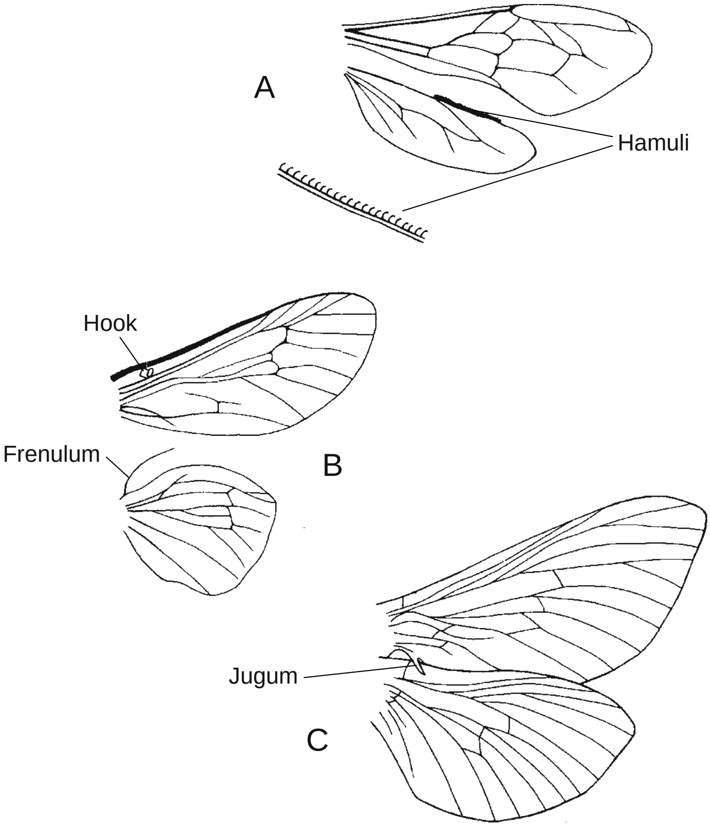

FIGURE 10.34. Mechanisms of wing overlap. A. The hamuli, a row of small hooks in Hymenoptera. B. The frenulum, a hair that protrudes from the posterior wing in some Lepidoptera. C. The jugum, a lobe that projects backward from the anterior wing in some Lepidoptera. From Romoser and Stoffolano (1998). Reprinted with permission.

development, the halteres arise from the second pair of wings because of the localized expression of the homeotic gene *Ultrabithorax* (*Ubx*). By experimentally inactivating *Ubx* from these cells, a second pair of fully formed wings results. With this change in a single gene, an insect's systematic status can be moved out of the order Diptera.

Various other proprioreceptors monitor the displacements of the body and wings during flight. In the locust, a wing hinge stretch receptor fires as the wing moves to the top of its range and changes its firing frequency with changing wing amplitude. A chordotonal organ responds to wing depression (Figure 10.37). These both modulate the motor neurons that activate flight muscles.

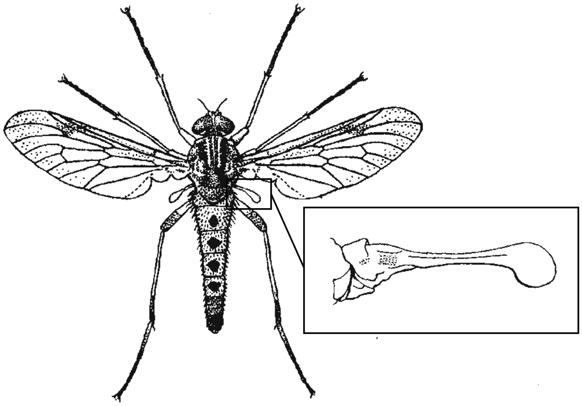

FIGURE 10.35. The second pair of wings has evolved into knoblike halteres in the Diptera. From Pringle (1975). Reprinted with permission.

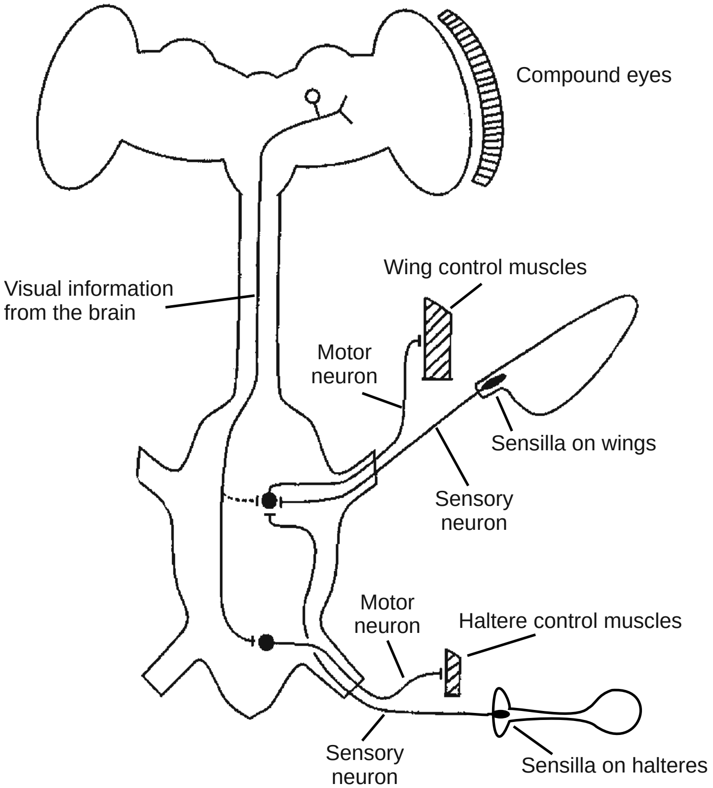

FIGURE 10.36. The mechanism of direct haltere control in the blow fly, *Calliphora*. The visual interneurons from the compound eyes activate the haltere control muscles. Twisting movements of the halteres activates their sensilla that feed to the wing muscle motor neurons and modulate their control. From Chan et al. (1998). Reprinted with permission.

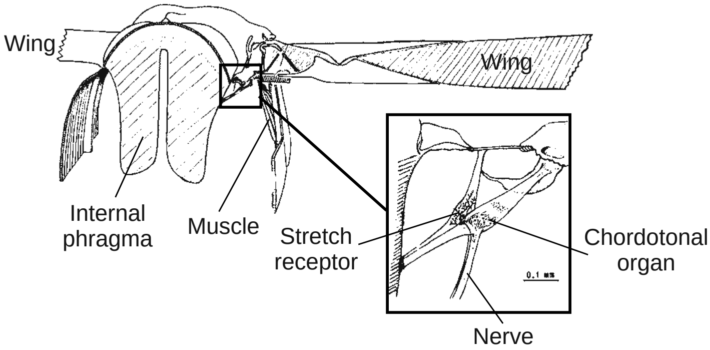

FIGURE 10.37. A chordotonal organ and stretch receptor associated with the wing hinge of the locust. The stretch receptor responds to the raising of the wing and the chordotonal organ to its lowering. From Möhl (1989). Reprinted with permission.

FLIGHT MUSCLE METABOLISM

Flight requires a relatively enormous amount of energy both because of the high cost of flying at a low Reynolds number and the inefficiency of the conversion of energy. Yet insect flight muscles operate completely aerobically. Only about 10% of the chemical energy used for flight is translated into mechanical energy, with the rest being dissipated as heat. This heat can be used for thermoregulation in some insects to allow them to fly even when ambient temperatures are too low for optimal muscle activity (see Chapter 7, Circulatory Systems).

The energy for flight muscle contraction is drawn from several sources (Figure 10.38). Ideally, these substrates would be situated within the muscles themselves to be close to where they would be utilized, but the need to maintain high ratios of muscle power to weight limits the concentrations they can attain there, necessitating that the bulk of the components be stored elsewhere. Small amounts of substances are located in the flight muscles themselves to power the initiation of flight, but the initial store of ATP in muscle cells is only sufficient for a few seconds of flight. It is replenished by the transfer of a phosphate group to ADP from **arginine phosphate**, providing an additional brief period of flight. This system is similar to vertebrate muscle where creatine phosphate is instead used as a reservoir for high-energy phosphoryl groups. The muscle may also store small amounts of other fuels including proline, glycogen, and triacylglycerol that are drawn upon during flight, but these resources are similarly limited.

Flight muscles draw the next most immediate source of energy from substrates in the hemolymph. The disaccharide trehalose is present in high concentrations as a circulating energy source that is used during the early phases of flight. Hemolymph diacylglycerol also bathes muscle cells, and the amino acid proline

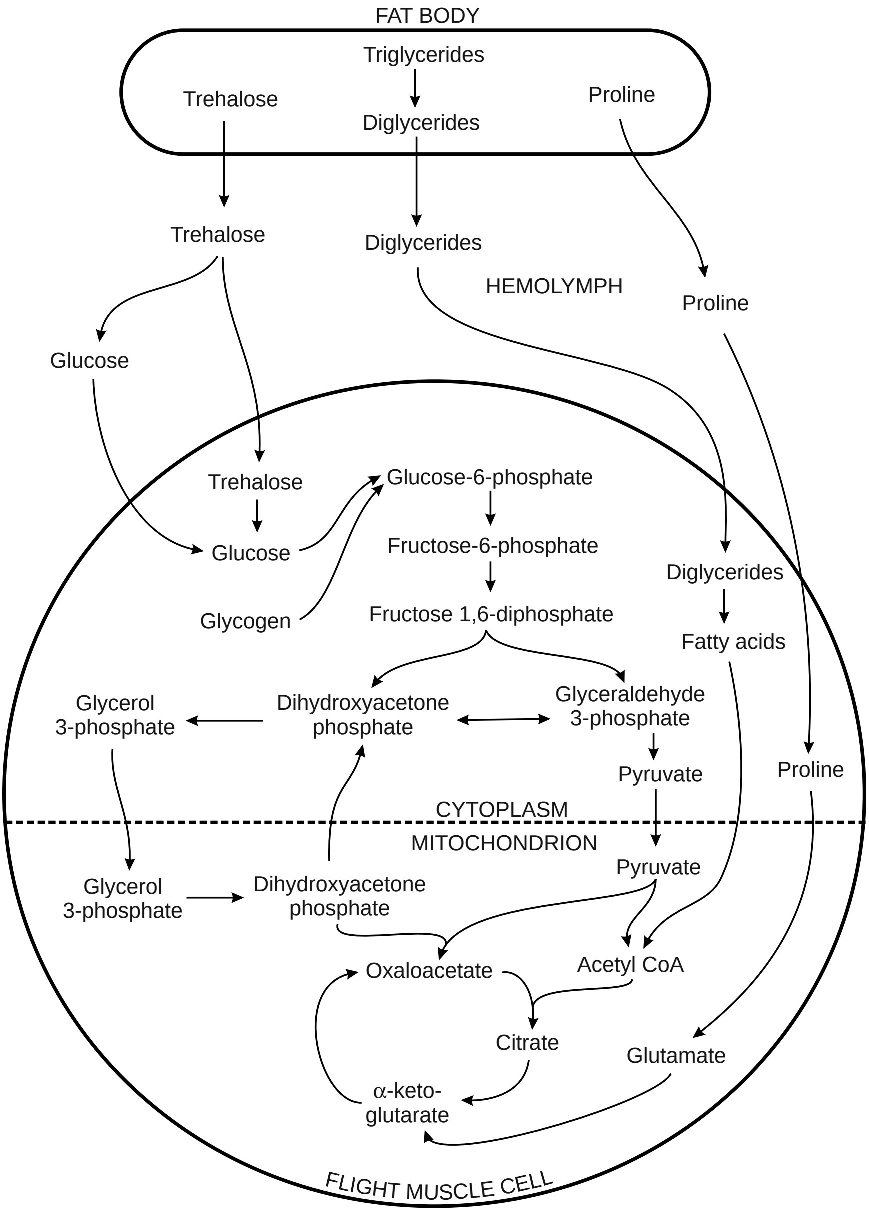

FIGURE 10.38. Utilization of various substrates from the fat body to fuel flight for muscle contraction. From Suarez et al. (2005).

is utilized in some insects for flight. These are mobilized from fat body reserves to maintain their levels in the hemolymph, but upper limits on the osmotic pressure of the hemolymph prevent the concentrations of these substances from getting too high.

The fuel for longer flights is stored in the fat body and transported to flight muscles through the hemolymph. This fuel use varies among insect orders. During long flights, dipterans and hymenopterans convert fat body glycogen into trehalose that is distributed to muscles through the hemolymph while migratory orthopterans utilize triacylglycerols in the fat body that are converted to diacylglycerols for transport.

Flight muscles completely oxidize carbohydrates to carbon dioxide and water in the absence of any anaerobic metabolism. Glycolysis in insect flight muscle occurs much like that in other animals, with a few additions. One is the presence of a glycerol phosphate shuttle, discussed in Chapter 6. With the conversion of glyceraldehyde phosphate to 1,3 diphosphoglycerate during glycolysis, NAD^+ acts as a hydrogen acceptor and is converted to NADH. Because the NAD^+ is only present in catalytic amounts, the reduced NADH must be reoxidized to maintain the biochemical pathway. In vertebrate muscle and insect muscles not involved in flight, NADH is oxidized during the conversion of pyruvate to lactic acid, and the liver or fat body rebuilds glucose as part of the oxygen debt. In insect flight muscle, no oxygen debt is incurred because the NADH is reoxidized by the enzyme glycerol phosphate dehydrogenase with dihydroxyacetone phosphate as a substrate to form glycerol-3-phosphate. Unlike NADH, the glycerol-3-phosphate can pass into the mitochondrion where it is reoxidized to form dihydroxyacetone phosphate. The dihydroxyacetone phosphate then leaves the mitochondrion to be oxidized by the NADH. Thus, the glycerol-3-phosphate shuttles the hydrogen into the mitochondrion where it can be harnessed for oxidative phosphorylation and also provide NAD^+ for glycolysis (Figure 10.39).

Dipterans use proline as fuel for flight to different extents. The blow fly, *Phormia regina*, initially metabolizes proline to provide the tricarboxylic acid intermediates that are used for later flight. However, the tsetse fly, *Glossina*, also uses proline, but as the major fuel for flight. As tsetse initiate flight, hemolymph proline decreases in concentration along with a concomitant increase in alanine concentrations and a brief increase in pyruvate during the first few seconds of flight. The pyruvate accumulates because it is produced faster than the rate of oxidation by mitochondria. These high levels of pyruvate initiate a series of biochemical steps beginning with the activation of the enzyme proline dehydrogenase that supplies tricarboxylic acid cycle intermediates to prime the cycle and speed up the rate of energy production. Glutamate is used to transaminate pyruvate so that proline enters the tricarboxylic acid cycle as α-ketoglutarate. The proline is not completely oxidized, losing two carbons to form alanine, which returns to the fat body and is reformed as proline by stored fatty acids. Thus,

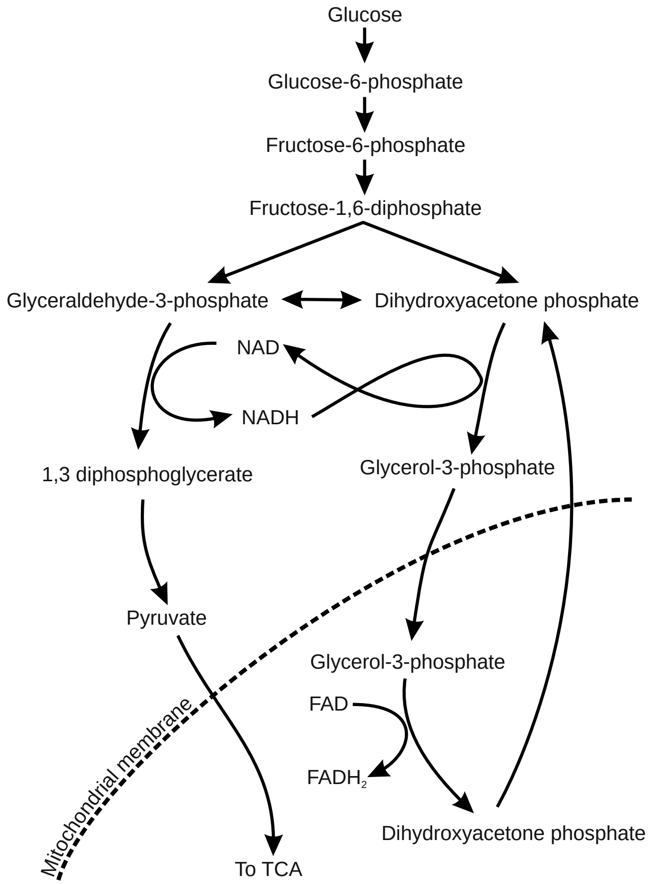

FIGURE 10.39. The glycerol-3-phosphate shuttle that operates in insect flight muscle.

the use of proline is actually a mechanism for shuttling two carbon units from the fat body to the flight muscles (Figure 10.40).

Lepidopterans may employ either lipids or carbohydrates, regulating their choice of fuels based on their feeding history. In general, insects that engage in long-range flights oxidize lipid, whereas those that use carbohydrate fly for only short periods. Insects with high wing-beat frequencies and asynchronous muscles tend to utilize carbohydrates, whereas those with synchronous flight muscles are more likely to utilize lipid. Lipid is the most concentrated form of energy storage. To fly 10 h each day, a migratory locust may use 70 mg of stored lipid. If glycogen were utilized instead, 500 mg or 30% of the insect's weight would have

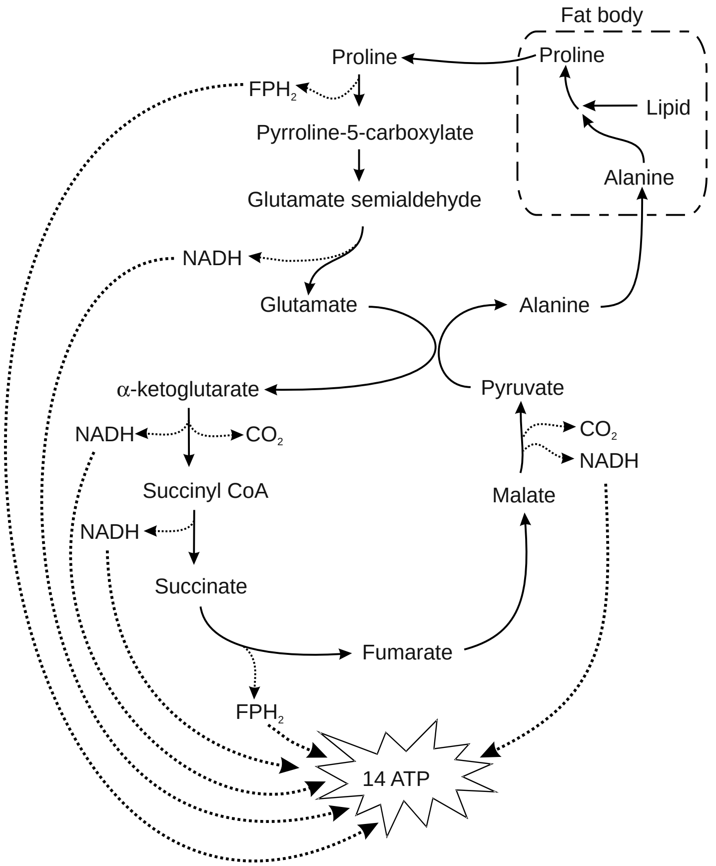

FIGURE 10.40. The utilization of proline as a fuel for flight.

to be oxidized. Glycogen is considerably more hydrated than lipid; as a result, it is also eight times heavier for the same caloric potential.

In spite of its many advantages, there are at least two drawbacks to the use of lipid to power flight. It is energetically expensive to interconvert ingested carbohydrates into lipids for storage and then back to carbohydrates for utilization. The net energy yield from a given carbohydrate is 20% higher when it is utilized directly instead of converted first to lipids. For this reason, many moths use the sugar directly when they have recently fed on nectar and draw on their fat reserves when starved. The other major drawback to storing energy as lipids is that they must be transported to flight muscles for use, yet they are insoluble in the aqueous transport medium of the hemolymph. A protein carrier molecule,

lipophorin, is produced by the fat body to shuttle the diacylglycerols to the flight muscles (Figure 6.33).

In locusts, energy metabolism during flight is initiated by octopamine and regulated by **adipokinetic hormone** (**AKH**). Trehalose serves as the major fuel at the onset of flight, but as the hemolymph trehalose levels decline with activity, octopaminergic neurons within the corpus cardiacum stimulate the release of AKH. The AKH activates an adenylate cyclase that increases cAMP levels and subsequently activates a protein kinase. The protein kinase then phosphorylates and activates a lipase that induces the release of diacylglycerols from the triacylglycerols stored in the fat body. AKH also induces the production of a lipoprotein carrier from the fat body that transports these diacylglycerols through the hemolymph to the flight muscles. The metabolism of carbohydrates that are stored in flight muscle during this lipid mobilization is also inhibited by AKH so that the lipid reserves are used exclusively. Octopamine has several other effects on flight behavior, stimulating the interneurons involved in maintaining flight, the power output of the flight muscles themselves, and the propriorecep-tors on the wing that monitor flight behavior. This amine may be the functional equivalent of flight-or-fight hormones in vertebrates, released during stress and causing an increase in the insect's arousal levels.

TERRESTRIAL LOCOMOTION

Insects possess a rigid exoskeleton that limits any significant bending except in specialized areas of joints and cuticular membranes. Single joints of the insect leg only allow movement to occur in a single plane, but more complex three-dimensional movements are possible given the arrangement of joints within the appendage. The limited movements that are possible may simplify the neural and muscular control of each appendage, but even with this relatively simple system, the act of walking, especially on uneven terrain, requires considerable motor skills. There are at least three joints in each leg that must be controlled, if the tarsi are neglected from consideration. These include the thorax-coxa joint, the coax-trochanter joint, and the femur-tibia joint (Figure 10.41). The presence of six walking legs presents 18 total joints that must be manipulated in order to walk. The larger number of legs compared to most vertebrates provides more opportunities for balance during locomotion but also presents challenges for coordination.

Motor programs that are encoded in the thoracic ganglia as **central pattern generators** send a set of muscle commands that coordinate leg movements and whose actions can be modified and adjusted by sensory inputs. These rhythmic motor patterns that trigger locomotion can be modified based on the information received from peripheral receptors. The pattern generators are located in the thoracic ganglia of each of the segments that bear legs. Each leg joint bears

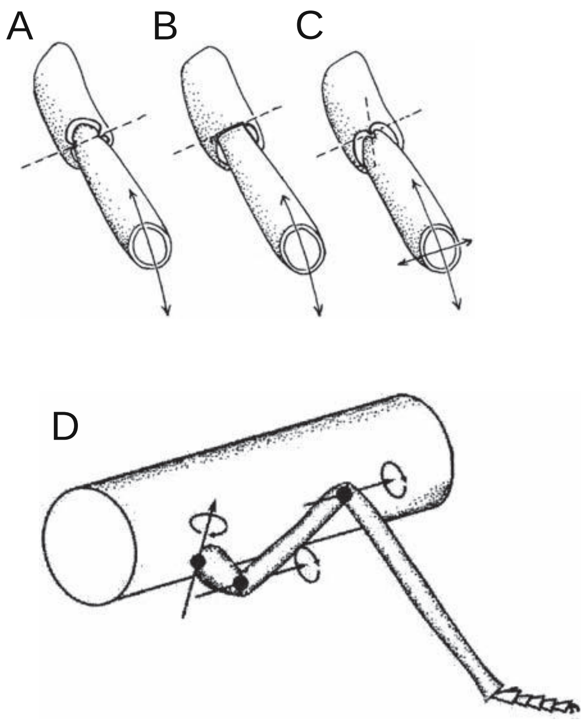

FIGURE 10.41. Types of arthropod joints. A. Pivot joint. B. Hinge joint. C. Monocondylar joint. D. Possible movements in space of an insect leg. From Wooton (1999). Reprinted with permission.

several mechanoreceptors that signal the joint's position, velocity, and acceleration to the central nervous system, allowing for immediate modification of the motor programs. This positional signaling permits the insect to sense that a leg no longer bears weight when it is supported by other legs and can therefore safely be moved. Each leg must have its own pattern generator for walking movements, and each pattern generator can be modified by changing external circumstances. These six pattern generators have equal status and are able to communicate with each other. The fine control of leg movements is possible by the presence of slow and fast axonal innervations. When slow walking is necessary, the firing of slow axons predominates, whereas at faster walking speeds, more fast axons are recruited. Feedback from both the internal and external environments modify the motor commands from the central nervous system that cause muscle contraction (Figure 10.42).

Humans use dynamic stability when we walk. When we fail to maintain our balance by constantly adjusting our muscles, we fall. Insects typically use **static stability**, in which the legs are positioned to maintain a stable configuration.

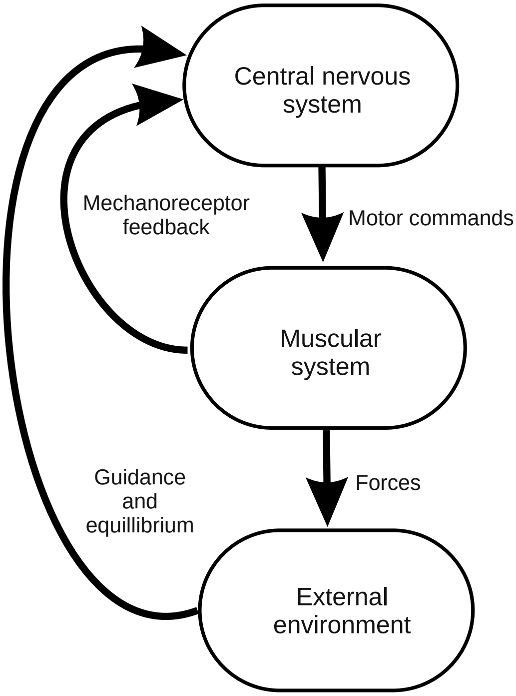

FIGURE 10.42. Mechanisms controlling muscle contractions that produce the movements associated with walking. From Dickinson et al. (2000). Reprinted with permission.

FIGURE 10.43. A cross section of the generalized insect body, suspended by the legs with a low center of gravity. From Delcomyn (1985). Reprinted with permission.

Legs both suspend and support the insect off the ground and provide the forces necessary to propel it forward. Being suspended by the legs provides the body with a low center of gravity and an unusual stability, but muscular activity is always required to keep the body off the substrate (Figure 10.43).

With their six legs, insects walk using an **alternating tripod gait** in which three legs, the middle on one side and the anterior and posterior legs on the other, alternate their contact with the substrate (Figure 10.44). This provides a tripod of stable support during walking, with the leg movements coordinated by

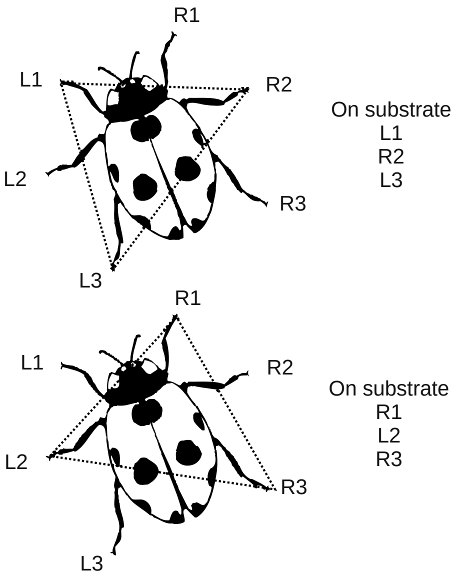

FIGURE 10.44. The alternating tripod gait of insects. The triangles show the legs in contact with the substrate.

the thoracic ganglia. Leg muscles are innervated by a mix of fast and slow neurons that can invoke a wide range of running speeds. The specific gait used often depends on the speed at which the insect is moving, with the tripod gait more likely to be employed at faster speeds and variable gaits, such as the **metachronal wave gait**, displayed at slower speeds. This gait involves a wave of leg movements on each side of the insect as seen in slow walkers. When an insect loses a leg, it adopts a different, more appropriate gait. Strictly speaking, insects do not run, if running is defined as movement in which all legs are no longer in contact with the surface, but a maximum progression of fast walking has been recorded for a number of insect species (Figure 10.45).

There are other unconventional insect walkers. Water striders are able to walk on water, not only remaining on the surface but capable of propelling themselves forward by using their legs as oars to create vortices in the water that impart momentum (Figure 10.46). Caterpillars walk by first moving their terminal prolegs forward and continuing with a body wave in which the muscles of each segment contract sequentially as the anterior prolegs are planted. The head moves forward as a result of hydraulic pressure. The biomechanical considerations inherent in a hydraulic skeleton and the inability to build momentum from previous steps limit these insects to a relatively slow speed. The limbless locomotion that

Insect	Speed (cm/s)
Bristletails	21
Cockroaches	18–80
Grasshoppers	4–15
Earwigs	10
Flies	9–20
Beetles	11–58
Ants	1–2.6
Caterpillars	0.3–3

Insect	Airspeed (m/s)
Grasshopper	3–13
Dragonfly	2–10
Butterfly	14
House fly	2–3
Horse fly	2–40
Mosquito	0.1–2
Honey bee	2–8
Tsetse	5

FIGURE 10.45. Average running and flying speeds of selected insects.

occurs in larval dipterans with hydrostatic skeletons that are also maintained by hemolymph pressure is about four times more energetically expensive than for a legged caterpillar of the same mass. They crawl using a combination of telescoping segments and peristaltic contractions (Figure 10.47).

The ability of some insects to walk on smooth vertical surfaces and even upside down is possible because their tarsi bear setae that secrete fluid droplets

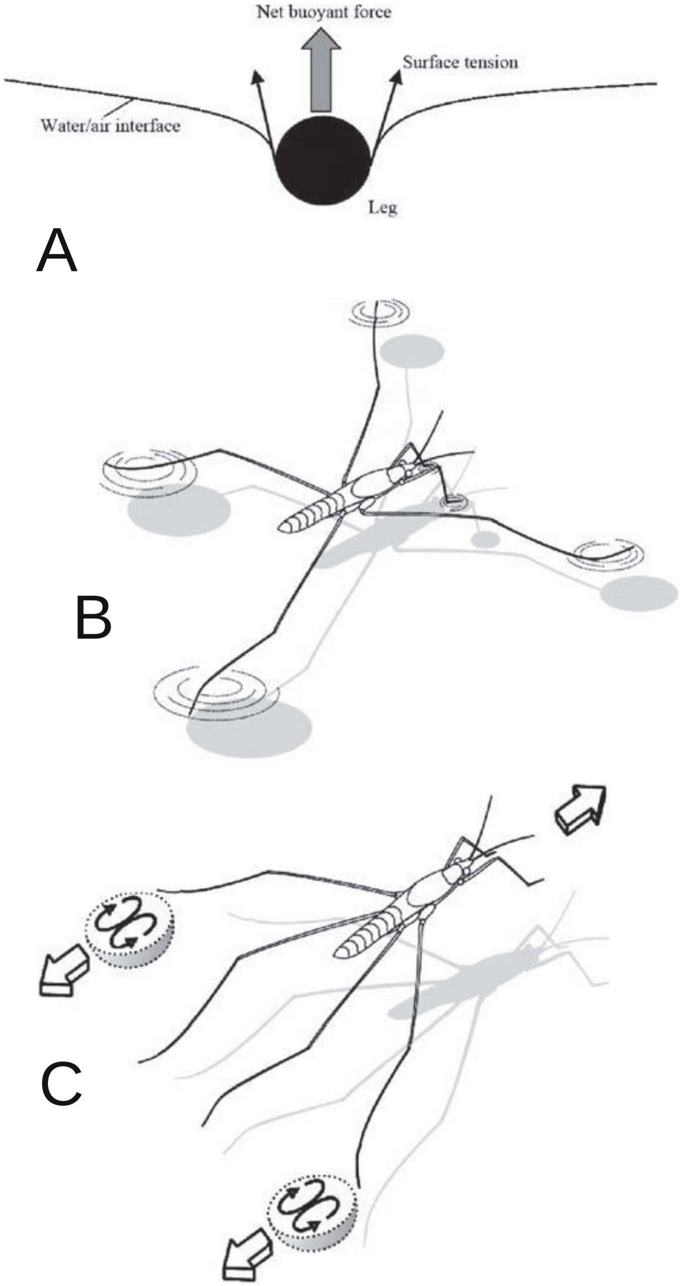

FIGURE 10.46. Water striders walking on the water surface. A. and B. Surface tension counteracts gravity at the water/air interface to keep the insect on the surface. C. Legs are used as oars to create vortices that propel the insect forward. From Denny (2004). Reprinted with permission.

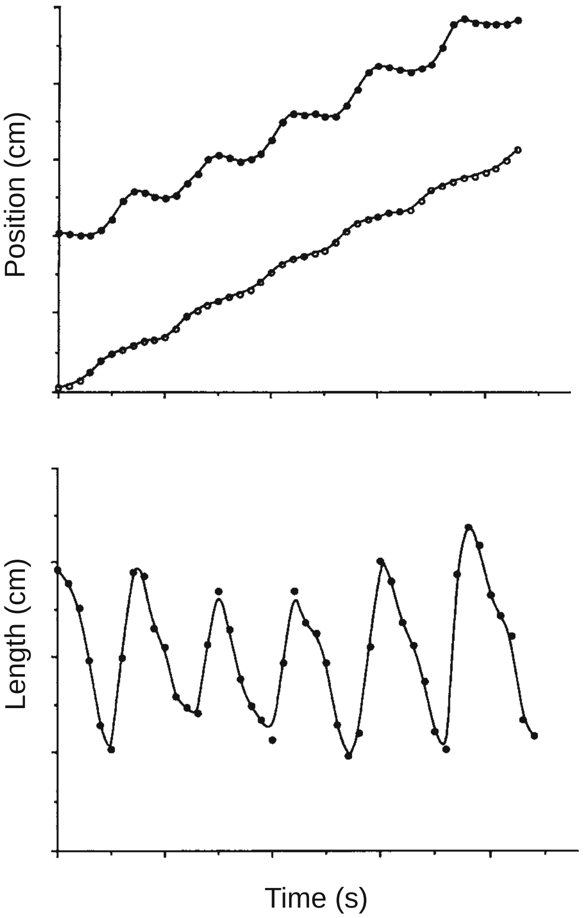

FIGURE 10.47. (*Top*) The movement of the head and rear of a larval *Drosophila* crawling in a straight line. (*Bottom*) The changes in body length of the same larva during locomotion. From Berrigan and Pepin (1995). Reprinted with permission.

that create capillary forces that cause the insect leg to stick. The pulvilli, which are large lateral lobes that arise from beneath the tarsal claws, are covered with setae that secrete adhesive substances from their terminal plates (Figure 10.48). The secretions of these plates, with a surface area of 2 μm by 1 μm, provide the adhesive properties of the footprint. Washing fly tarsi with solutions that dissolve these secretions significantly reduces the attractive forces.

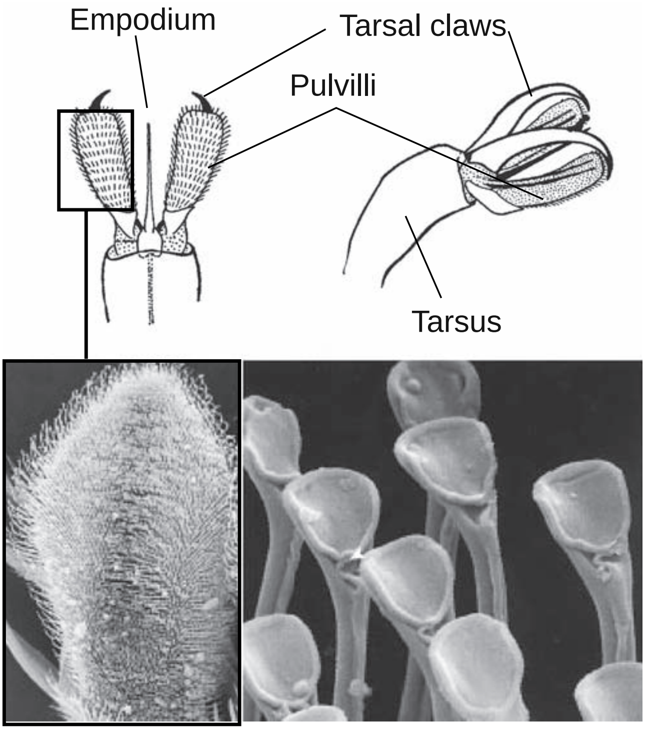

FIGURE 10.48. The hairs on a typical insect tarsus that secrete adhesive fluid that allows them to walk up smooth vertical surfaces. From Snodgrass (1935) and Gorb and Beutel (2001). Reprinted with permission.

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Nervous Systems

We have already described the endocrine system, used to process information and coordinate the activities of insect cells. Hormones, the chemical signals released by endocrine centers, can ultimately communicate with every cell by traveling through the circulatory system. However, the chemical messages are relatively slow in arriving because they depend on diffusion through the hemolymph for transport. An alternative is for information to be transmitted by the electrical signals of the nervous system, providing a much more rapid means for coordinating cellular events.

If biological information is to be transferred in a timely manner, the nervous system is certainly the preferred route. Electrical signals can reach a distant part of the insect within milliseconds, compared to the minutes or hours that it might take a hormone to do the same. If the insect discovers food or is threatened by a predator, the signals that are sent to the muscles that move the animal toward or away from the stimuli are best transmitted by nerves. By the time a hormonal signal was sent and received, the food might be gone and the predator might already have made a meal of the insect. An extensive hard-wired communication system takes up precious space, however, and a relatively small animal cannot afford to have too much space taken up by these lines to each effector organ. Insects use both the endocrine and the nervous systems for information transfer

between their cells, with each system having its own advantages and constraints. This chapter examines aspects of nervous transmission.

BASIC COMPONENTS OF THE NERVOUS SYSTEM

The Neuron

Nerve cells, or **neurons**, are the cellular building blocks that make up the nervous system. They are capable of integrating information, undergoing excitation, and transmitting the information by electrical and short-range chemical signaling. They arise from the ventral epithelium during embryogenesis as **neuroblasts** that develop from gene expression by a cluster of **proneural cells**. As neural stem cells, the neuroblasts delaminate from this neuroectoderm and divide asymmetrically to produce ganglionic mother cells that each then divide to form both glial support cells and neurons.

The basic structure of a neuron consists of a cell body, called a **soma** or **perikaryon**, which contains the nucleus, and its projections that form the axons and dendrites (Figure 11.1). All protein synthesis occurs in the soma, and metabolites synthesized there are transported to the neural processes. The soma contains a large nucleus and an abundance of Golgi complexes and rough endoplasmic reticulum, whereas these organelles are generally absent from the axons, the processes that typically transmit information to other neurons. Axons carry information away from the cell and often contain smooth endoplasmic reticulum and neurosecretory vesicles. Branches from the axons make up the dendrites, regions that are specialized to receive information from the neuron. Unlike those of vertebrates, most insect neurons are **monopolar** and bear a single axon from the soma, but those involved with peripheral receptors may be **bipolar**, and internal stretch receptors are often **multipolar** (Figure 11.1). What are referred to as "nerves" are bundles of axons commonly surrounded by sheaths that arise from the cell bodies that are located within the ganglia.

They may be distinguished according to their function as **sensory neurons**, **motor neurons**, **interneurons**, or **neurosecretory neurons** (Figure 11.2). Sensory neurons carry messages from sensory receptors, and motor neurons regulate the contraction of muscles. The connections between sensory and motor neurons are usually mediated by the interneurons that represent the bulk of the nerve cells in the central nervous system. The aggregated soma of motor neurons and interneurons make up the **ganglia** of the insect central nervous system, with the soma of sensory neurons generally located not in the ganglion but near the receptor itself. Within the central **neuropil** of the ganglion are the axons, dendrites, and synapses of neurons, whereas the cell bodies of motor neurons and interneurons are situated peripherally (Figure 11.3). Neurosecretory cells are

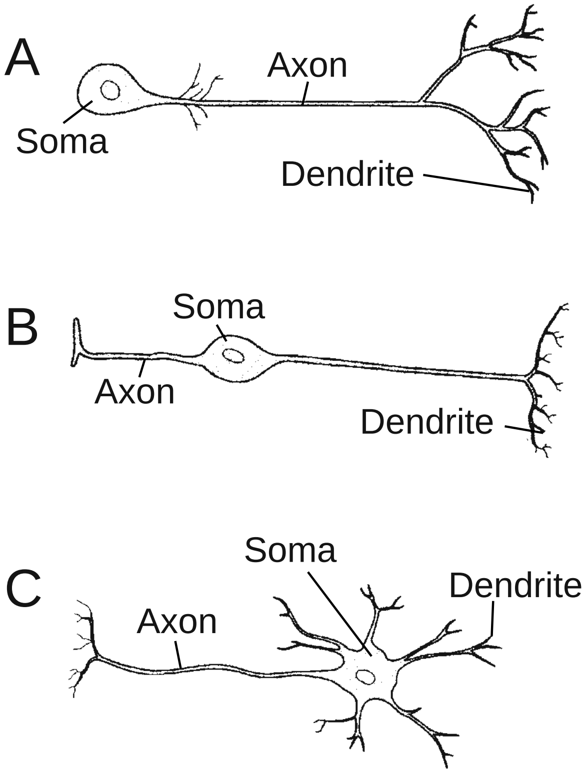

FIGURE 11.1. Examples of insect neurons. A. Monopolar. B. Bipolar. C. Multipolar.

neurons that release their neurosecretory products into the circulatory system and are discussed in Chapter 1.

Glial Cells

A system of barriers is present to maintain the chemical environment of the neurons separately from other tissues. A complex network of membranes and intercellular channels surrounds the nerve cells to maintain the ionic differences that are responsible for the electrical potential that is required for the nervous system to operate. Each neuron is almost completely surrounded by sheath material secreted by numerous **glial cells** (glia, Greek for glue) that insulates and provides it with nutrients. The sheaths are only absent at the synapse to allow the neurons to interact. Because the hemolymph of many insects is high in potassium and low in sodium, an environment that conflicts with the operation of the nervous system, a blood-brain barrier is necessary to isolate the neurons.

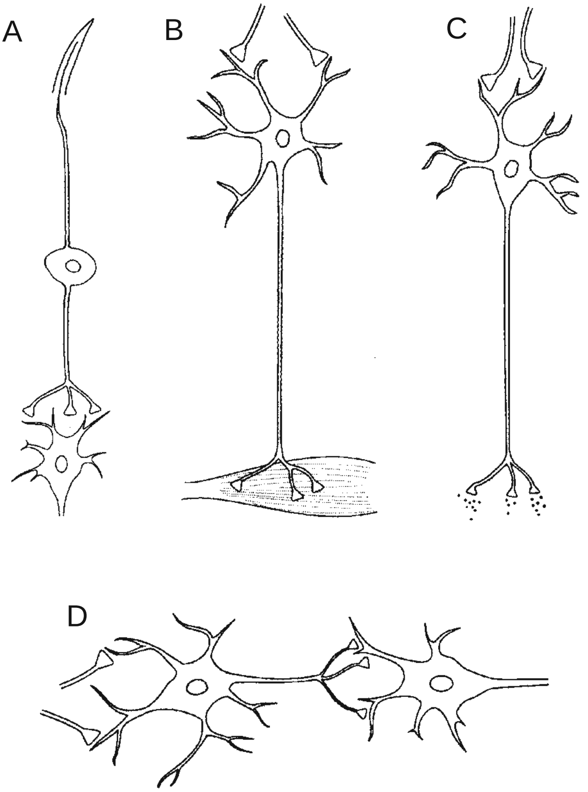

FIGURE 11.2. Types of neurons. A. Sensory. B. Motor. C. Neurosecretory. D. Interneuron.

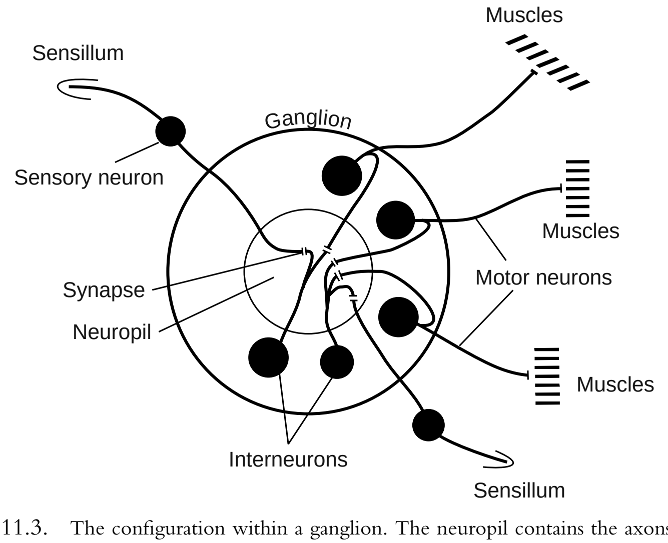

FIGURE 11.3. The configuration within a ganglion. The neuropil contains the axons, dendrites, and synapses, with their soma located along the outside of the ganglion core. The soma of sensory neurons are located near the receptor.

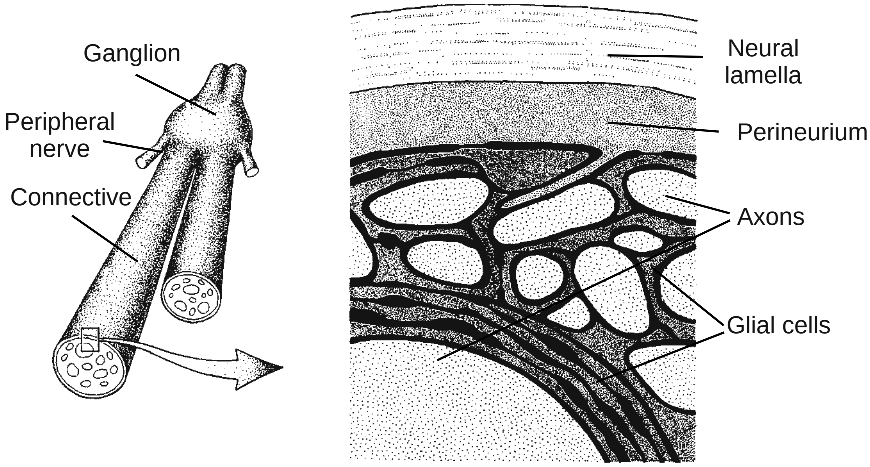

FIGURE 11.4. (*Left*) The connectives of the central nervous system of the cockroach. (*Right*) The axons are enclosed by the perineurium, a layer of glial cells, and overlaid by the neural lamella. From Treherne (1985). Reprinted with permission.

The glial cells are instrumental in maintaining the controlled environment of the neuron using ion pumps to regulate the movement of sodium from the hemolymph to the extracellular fluid located in the channels formed by these cells. An additional outer layer of specialized glial sheath cells forms the **perineurium** that secretes the outermost **neural lamella.** The lamella surrounds the entire nervous system and is largely responsible for this blood-brain barrier in insects. The perineurial layer and the lamella are collectively referred to as the **nerve sheath** (Figure 11.4). The presence of glycogen within the cells associated with the perineurial layer and the changes in its accumulations suggest that these cells are involved in the transfer of metabolites to the neurons.

During embryonic development of the nervous system and its remodeling during metamorphosis, glial cells provide both attractive and repulsive cues for the guidance of neuronal growth. Glia along the developmental midline provide the cues that guide axonal growth outward from the ventral nerve cord, and those located in the eye imaginal disc present photoreceptor axons with cues that determine their targets within the structure of the compound eye. Glial cells in the mushroom body of the brain are signaled by 20HE to engulf and degrade the degenerating axons that already have been targeted for death during metamorphosis.

Maintenance of Electrical Potential and Nervous Transmission

All living cells actively engage in ion transport across their cell membranes, resulting in an electrical potential difference that makes the inside of the cell

more negative than the outside. Generally, potassium is taken up, whereas sodium, magnesium, and calcium are pumped out. Neurons differ from other cells in that this electrical potential is able to vary substantially. The difference in electrical potential, or polarization, between the inside and outside of an unstimulated neuron is approximately −70 mV. This polarization, maintained by a sodium/potassium pump in the absence of any nervous transmission, is called the **resting potential**.

In the process of **sensory transduction**, sensory receptors convert energy from the environment into electrical energy. Light, mechanical deformation, or chemical signals cause the dendrites of the sensory receptors to undergo a depolarization that is proportional to the amount of stimulation. This variable change in electrical potential in the dendrite after stimulation is called the **receptor potential**. The generation of a receptor potential in turn causes the all-or-nothing depolarization along the axon known as an **action potential** that sweeps along the axon to the terminal arborizations at the synapse. The action potential in any one location in the axon lasts for 1 to 2 milliseconds before the ion pumps restore it, but the depolarization moves down the axon until it reaches the synapse.

Events Occurring at the Synapse

At the terminal end of the axon, the electrical energy that was propagated must be converted to chemical energy in order to stimulate the neighboring neuron. When the action potential arrives at the presynaptic membrane, ion channels open that cause calcium to enter the neuron and stimulate synaptic vesicles to fuse with the membrane. The fusion of the vesicles causes the release by exocytosis of the neurotransmitters into the **synaptic cleft**, a space that varies between 20 and 50 nm in width. When more frequent depolarizations reach the presynaptic membrane, there is a greater fusion of vesicles and the release of more neurotransmitters. The neurotransmitters diffuse across the synaptic cleft, where they may reversibly bind to specific receptors on the postsynaptic membrane and induce a conformational change that alters the permeability of the membrane and induces its subsequent depolarization. This postsynaptic membrane is surrounded by a dendritic membrane that ultimately joins the axon and propagates this depolarization into another action potential that moves along the axon of the second neuron (Figure 11.5).

A number of different neurotransmitters and neuromodulators are involved in nervous transmission at both invertebrate and vertebrate synapses (Figure 11.6). They are believed to have first appeared about 1 billion years ago and have remained as the major signal molecules across the animal kingdom. The most common excitatory neurotransmitter is **acetylcholine**, released by interneurons in the neuropil and by sensory receptors. The amino acid **glutamate**

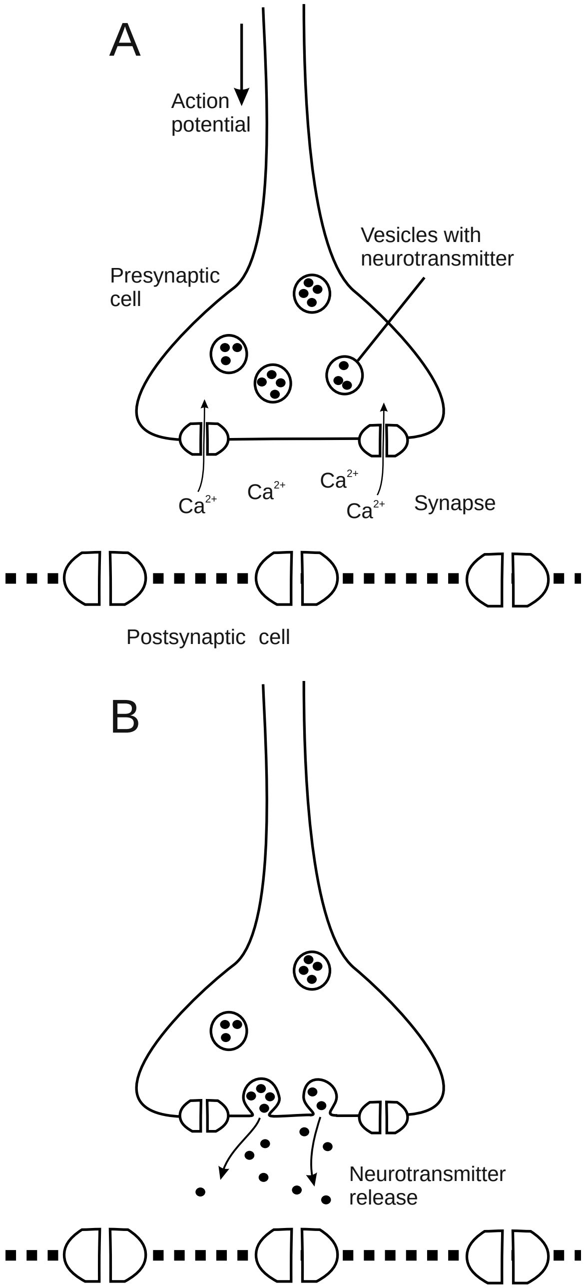

FIGURE 11.5. Events at the synapse. A. The change in membrane potential arriving at the presynapse as an action potential causes the uptake of calcium and the release of neurotransmitter stored in vesicles. B. The neurotransmitter is released into the synapse.

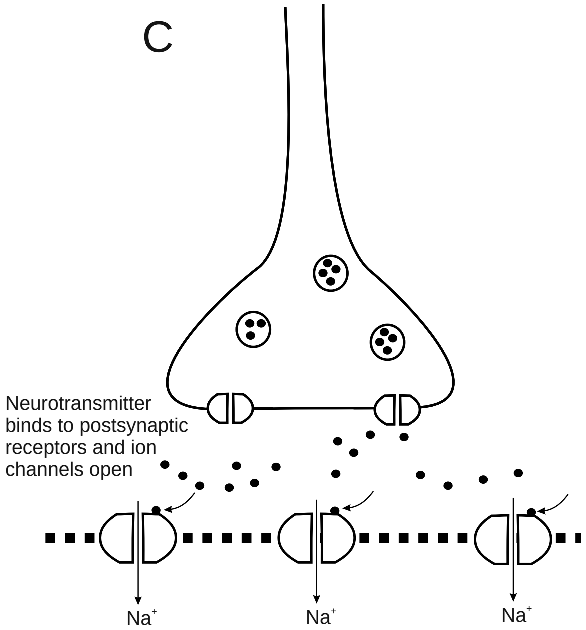

FIGURE 11.5. *Continued* C. It binds to receptors on the postsynaptic membrane, leading to a change in membrane potential of the postsynaptic neuron.

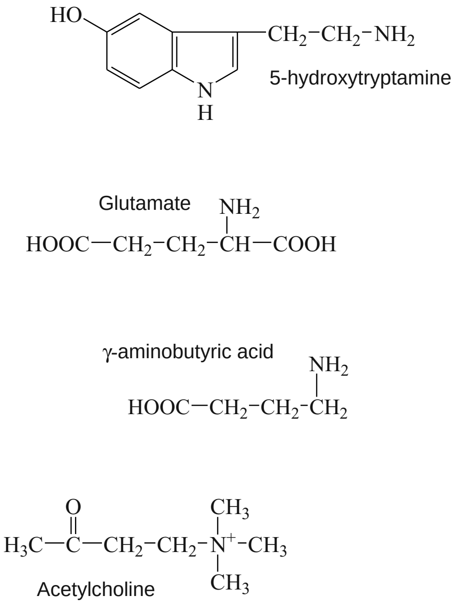

FIGURE 11.6. Common insect neurotransmitters and neuromodulators.

is the principal excitatory neurotransmitter at the neuromuscular junction. Some synapses contain neurotransmitters that inhibit rather than stimulate the postsynaptic membrane. **Gamma-aminobutyric acid** (**GABA**) is an inhibitory neurotransmitter in both the neuropil and neuromuscular junction. Neuromodulators like GABA may affect either the release of a neurotransmitter from the presynaptic membrane or the response of the postsynaptic membrane to other released neurotransmitters. Neuropeptide transmitters include **proctolin**, several **tachykinins**, and the **FMRFamide-related peptides**. **Nitric oxide** (NO) is a second messenger in insect systems and also serves as a neurotransmitter, as it does in mammalian systems. NO synthase is present in insect brains, and the NO precursor L-arginine is required for memory function in mantids. NO may thus be a neurotransmitter involved with learning and memory formation.

Several biogenic amines function in neuromodulation, influencing the function of neurons but not acting as a transmitter. These include **histamine**, **dopamine**, **serotonin**, **tyramine**, and **octopamine** (Figure 11.7). Octopamine, structurally related to noradrenaline, is noteworthy because of its multifunctional role in modulating the responses of flight muscles and sensory receptors and also acting as a hormone when it is released into the hemolymph. Octopamine is involved in the modulation of almost every physiological process that has been studied so far in invertebrates. Its targets are sense organs and processes within the central nervous system, desensitizing sensory inputs and in general regulating the animal's mood and response patterns. A group of octopaminergic neurons known as **dorsal unpaired median neurons** (**DUM neurons**) is found in all segmental ganglia. These innervate skeletal and visceral muscles as well as endocrine glands and sensilla, and they modulate their responses. Some DUM neurons form neurohemal organs to release octopamine over muscles and into the hemolymph. Tyramine is an intermediate product of the synthesis of octopamine from tyrosine and consequently is produced in all octopaminergic neurons. Little is known about the role of tyramine in insect systems other than its opposite effects compared to octopamine. Whereas octopamine increases second messenger levels of target cells and enhances muscle contraction, tyramine causes them both to decline. Tyramine is also produced and released by stellate cells of the Malpighian tubules, and when taken up by principal cells, it induces increased chloride conductance.

Enzymes within the synaptic cleft degrade the neurotransmitters after they have been bound and allow some of these materials to be recycled to the presynaptic neuron for resynthesis of neurotransmitters in the vesicles. This degradation also prevents the neurons from being continually activated by a stimulus that is no longer present and opens the receptor to stimulation by a subsequent release of neurotransmitters. Carbamate and organophosphate insecticides act by inhibiting the enzyme acetylcholine esterase and preventing the degradation of acetylcholine at the synapse, thus interfering with nervous transmission and coordination within the insect.

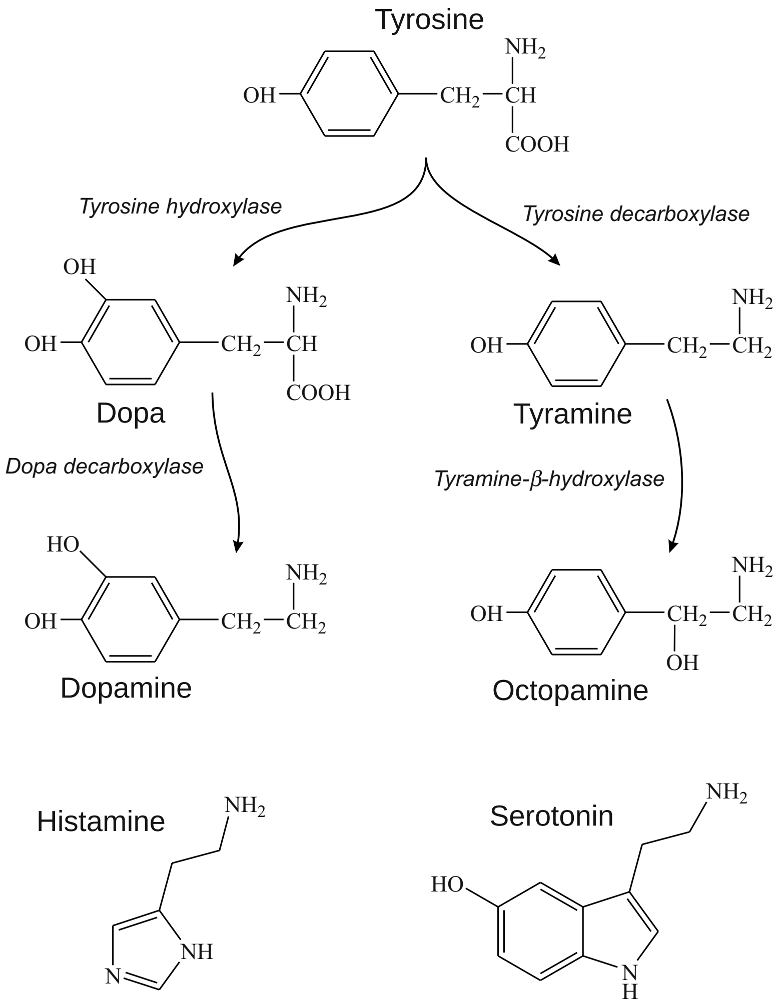

FIGURE 11.7. Synthesis of biogenic amines from tyrosine.

The synaptic connection of a single neuron with two or more neurons, or with a muscle cell innervated by multiple motor neurons that includes neurotransmitters capable of exciting or inhibiting at the synapse, allows the nervous system to integrate nervous transmission in a much more complex fashion (Figure 11.8). In the cockroach cercus, for example, a few hundred mechanoreceptors converge on an interneuron that mediates the escape response. When the neurotransmitter that is released by all the sensory cells is the same, an additive excitation or inhibition can be created. When excitatory and inhibitory connections are mixed, the response can be a total inhibition or excitation at the synapse

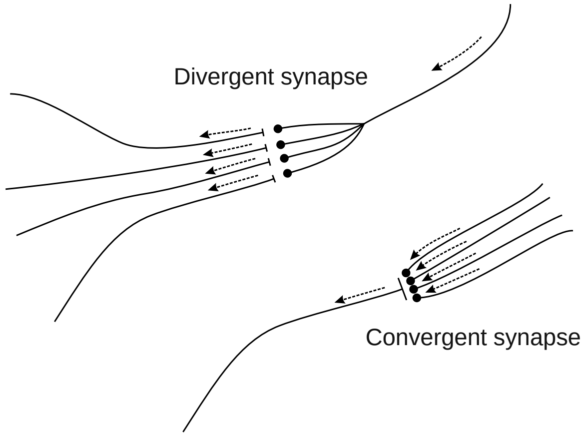

FIGURE 11.8. A divergent synapse, where one axon stimulates several dendrites, and a convergent synapse, with several axons from different neurons converging on a single dendrite.

or a partial effect. Divergent connections that involve a single neuron stimulating two or more different interneurons can take a message from a single receptor and spread it to several cells. There may also be loops that provide feedback to modulate the output of a motor or sensory neuron.

EVOLUTION AND STRUCTURE OF THE NERVOUS SYSTEM

The Central Nervous System

The insect central nervous system consists of the **brain** and **ventral nerve cord**. The system comprises the individual neurons that are associated in functional groupings that evolved together with a reorganization of the insect body from its primitive annelid-like ancestor. Neurons evolved from ectodermal cells that assumed the properties of irritability and conductivity, and they were ancestrally arranged in two lateral bands of ectodermal nerve cords that ultimately moved together and fused laterally (Figure 11.9). The primitive nerve mass at the anterior united the cords and served as the primitive brain or **archicerebrum**. The extensive reorganization of the nervous components that resided in the anterior body segments followed the consolidation of the anterior segments and their appendages into the head capsule and its mouthparts.

The insect brain comprises a large grouping of neurons that lies above the esophagus, and for that reason it has also been referred to as the **supraesophageal ganglion** (Figure 11.10). It is a composite structure derived in part from

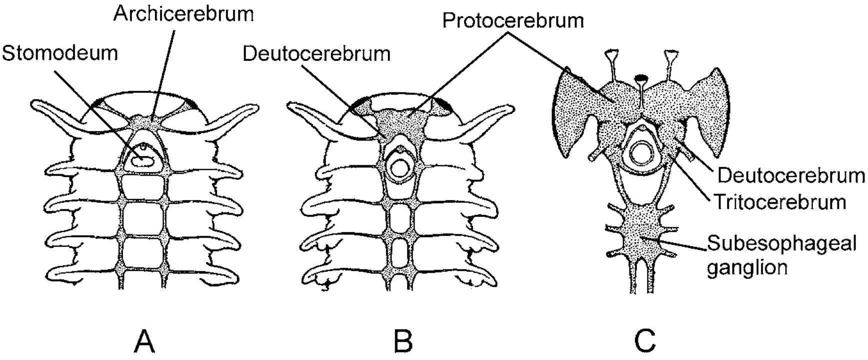

FIGURE 11.9. Possible evolution of the brain and central nervous system from an annelid-like ancestor. A. The primitive archicerebrum originated as a mass of ganglia that united the two nerve cords. B. The specialization of the protocerebrum and deutocerebrum into ocular and antennal centers. C. The present-day insect brain, with its protocerebral, deutocerebral, and tritocerebral lobes, and the subesophageal ganglion that arose from the consolidation of the next three ganglia. From Snodgrass (1935). Reprinted with permission.

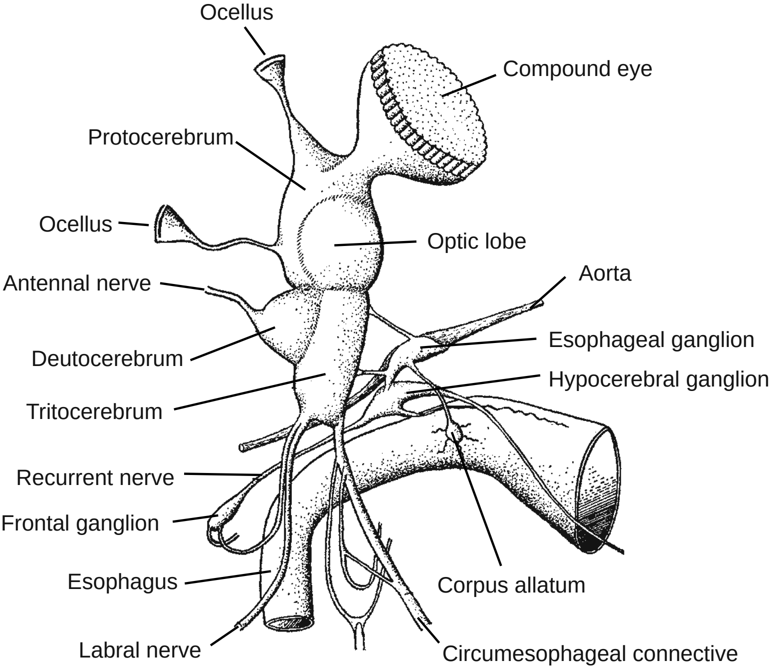

FIGURE 11.10. A side view of the structure of the insect brain, sitting atop the foregut. From Albrecht (1953). Reprinted with permission.

the primitive archicerebrum of its annelid ancestors, but there is not total agreement about the origin of its current form. The precise number of primitive segments that constitute the insect head is still a matter of controversy. The three sections of the brain — the **protocerebrum**, **deutocerebrum**, and **tritocerebrum** — may have each originated from a different segment of the primitive head capsule, although some believe that the deutocerebrum may have evolved as an antennal lobe of the original protocerebrum. The number of neurons in the brain of insects varies from about 1.2 million in the cockroach, 950,000 in the honeybee, and 200,000 in *Drosophila*.

The protocerebrum is associated with the compound eyes, ocelli, and some integumental sensory receptors. It consists of two large lateral lobes containing dense groupings of neurons and neurosecretory cells within the area known as the **pars intercerebralis** along the anteriodorsal midline. The neurosecretory cells transport neurosecretory material to the corpora cardiaca and corpora allata, where it is released. The **glomeruli** are areas of the brain that are dense with synaptic contacts. Those glomeruli lateral to the pars intercerebralis are the **corpora pedunculata**, or **mushroom bodies**, each consisting of a dorsal **calyx**, or head, that rests on a stalk, the **pedunculus**. Intrinsic neurons that densely make up the mushroom bodies are called **Kenyon cells**, known for their characteristic arborization of neural processes. A number of differing subpopulations of Kenyon cells have been identified, but their functional correlates are still unknown. The honeybee brain contains at least three Kenyon cell subpopulations that vary in dendritic and axonal morphologies and the neurotransmitters they utilize. They receive abundant inputs from sensory neuropils, including those of the antennal lobes, as well as mechanosensory and gustatory inputs from the tritocerebrum, and they are believed to be essential for processing olfactory information and the storage of olfactory memory. They have also been implicated in the control of tasks that require visual coordination of locomotor activity and spatial orientation. The mushroom body and its unique Kenyon cells form a system of neurons that provides a structure for elaborate interconnections in the brain.

Lateral to the corpora pedunculata are the **optic lobes**, the portion of the protocerebrum that extends to the compound eyes. Each consists of three nested groups of neuropils that process the sensory information from the compound eyes. Direct inputs from the photoreceptors of the compound eye are received by the **lamina**, the outermost of the neuropils, and is connected to the **medulla** and the innermost **lobula complex**. Figure 11.11 outlines some of the major neuropils of the honeybee brain.

The deutocerebrum innervates the sensory receptors and muscles of the antennae. It, too, is divided into a series of glomeruli, the dense areas of neuropil surrounded by glial cells in which many synapses occur. These are areas of neural convergence where hundreds of thousands of receptor neurons synapse with several hundred interneurons, with these in turn synapsing with other neurons

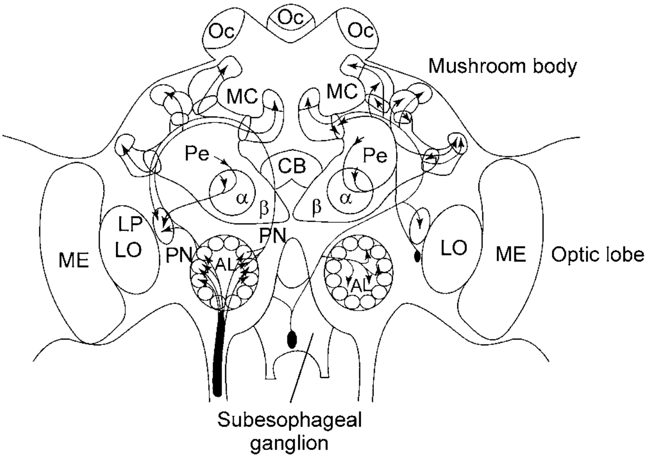

FIGURE 11.11. Glomeruli within the honeybee brain. The major neuropils include the antennal lobe (AL) and the mushroom body (MB), which consists of the calyx (MC, medial calyx, LC, lateral calyx), whose axons form the pendunculus (Pe). The α and β lobes are the output regions of the mushroom body. The lateral horn (LP) is another olfactory neuropil within the lateral protocerebrum. The PN (projection neurons) comprises two neural tracts that communicate between the AL and the MB. The medulla (ME) and lobula (LO) are visual neuropils. Oc, Ocelli; CB, central body. From Menzel and Giurfa (2001). Reprinted with permission.

that project into the mushroom bodies of the protocerebrum. The number of glomeruli varies among insects; locusts contains about 1000 glomeruli, and about 125 are present in American cockroaches. Each glomerulus occupies a constant position and receives specific sensory neurons, suggesting that each may have a separate functional identity. The neurons within these glomeruli respond when antennal receptors are activated by pheromone blends or food odors.

The tritocerebrum lies beneath the deutocerebrum and is the third and smallest part of the brain. It connects the central nervous system to the visceral nervous system through the frontal ganglion, and to the ventral nerve cord through the **circumesophageal connectives**. It also receives nerves from the labrum. Relatively little else is known about its functions.

Each of the other body segments primitively contained a ganglion, a group of nerve cells that was responsible for the activities of that segment. Each ganglion may contain from a few hundred to several thousand neurons that integrate the inputs from sensory receptors and control the muscles within the segement. The **subesophageal ganglion** is the first ganglion of the ventral nerve cord and the only one in the head. It is a compound ganglion formed by the fusion of the ganglia of the mandibular, maxillary, and labial segments. It gives off paired nerves to each of the mouthpart appendages and innervates the salivary glands. The remainder of the ventral nerve cord consists of a series of paired ganglia,

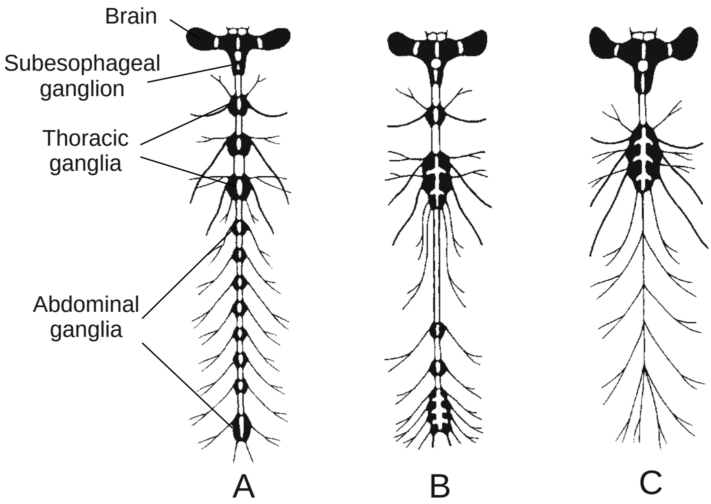

FIGURE 11.12. Consolidation of the ventral ganglia. A. Example in a coleopteran. B. Example in a hymenopteran. C. Example in a dipteran. From Gullan and Cranston (2000). Reprinted with permission.

joined by lateral connectives, which contain the perikarya of motor neurons and interneurons. The first three are in the thorax, serving as a locomotor center that controls the wings and the legs. In some insects, these ganglia are consolidated to form a compound **metathoracic ganglion**. Primitive thysanurans have eight abdominal ganglia, but the evolutionary trend has been toward a reduction in the number of abdominal ganglia and most insects have fewer (Figure 11.12). The metathoracic ganglion may be fused with the first one to three abdominal ganglia, and the seventh and eighth abdominal ganglia are commonly fused to form the compound **terminal abdominal ganglion**. This terminal abdominal ganglion innervates the hindgut and reproductive structures and regulates oviposition behavior in females and the mating behavior of both sexes.

The **peripheral nervous system** involves all the nerves that radiate from the central nervous system. These include the nerves that innervate muscles, stretch receptors that may serve as proprioreceptors, innervations of the reproductive system and spiracles, and the various sensory receptors.

THE VISCERAL NERVOUS SYSTEM

The portion of the nervous system described so far is largely devoted to moving the insect around and mediating interactions with the external environment. Another part of the insect nervous system is concerned with maintaining the

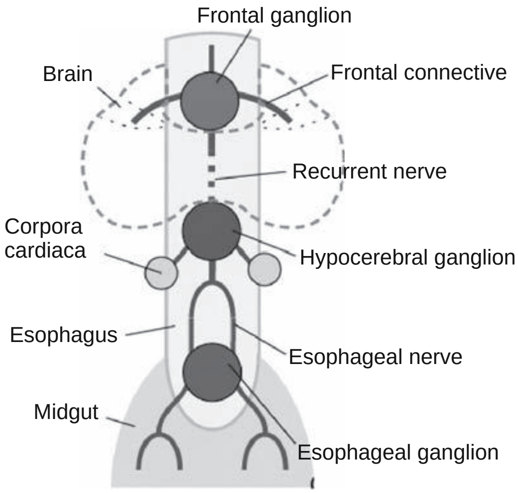

FIGURE 11.13. The stomatogastric nervous system. From Hartenstein (1997). Reprinted with permission.

internal environment and coordinating various internal functions, much like the autonomic nervous system of vertebrates. This element of the nervous system, termed the **visceral nervous system**, innervates the gut, heart, and endocrine glands and forms a network of peripheral ganglia that is associated with digestive processes. These ganglia innervate the muscles of the oral cavity, foregut, and midgut and regulate food ingestion, transport, digestion, and the excretion of wastes. The **stomatogastric nervous system** is that part of the visceral nervous system that specifically innervates the foregut and midgut. The major ganglion of the visceral nervous system is the **frontal ganglion** that arises from the frontal connectives issued by the tritocerebrum. The frontal ganglion innervates the foregut and controls crop emptying. It gives rise to the **recurrent nerve** that passes underneath the brain and expands into the **hypocerebral ganglion** that innervates the corpora allata, corpora cardiaca, and the fore- and midguts (Figures 11.10 and 11.13). The **caudal autonomic system** consists of those nerves that innervate the hindgut and genitalia, usually originating in the compound terminal abdominal ganglion.

SENSING THE ENVIRONMENT

The insect integument provides an effective barrier against water loss and environmental assault. It separates the insect from its environment and allows the proper internal conditions to be maintained. However, this separation can also

interfere with the detection of changes in the environment that allows the insect to make biologically appropriate responses. Chemical messages such as pheromones mediate mate location, the volatiles from host plants and animals identify food and oviposition sites, and various signals from other individuals must trigger alarm and aggregation responses. The nervous system, residing on the inside of the impenetrable integument, must be able to receive information across this barrier. To allow this to occur, a compromise had to be struck between the need for the insect to conserve water and the need to sample the environment with a biological membrane that by necessity must contain a moist receptor surface.

Insects have addressed this problem by allowing these receptor surfaces to be exposed only through extremely small pores. The pores are open continuously, but their small size minimizes the potential for water loss. Receptors are also not distributed uniformly over the body but are concentrated on a few areas that would be most likely to receive stimuli, such as the mouthparts, antennae, legs, and cerci, allowing most of the insect body to remain waterproof but also insensitive to external stimuli.

Another unique property of the sensory receptors of insects is that they are all **primary sense cells** instead of the secondary sense cells that respond to taste, touch, and vision in vertebrates. A secondary sense cell is a cell of non-neural origin that is linked to a neuron. Vertebrate touch receptors in the skin are modified epidermal cells that produce a receptor potential and then relay that potential to a neuron. When the cell that receives environmental stimuli is a bipolar primary sense cell, it produces both a receptor potential and an action potential, and there is no need for the second neuron (Figure 11.14). One cell in insects performs the same function as two do in vertebrates: the primary sense cell and the neuron that innervates it.

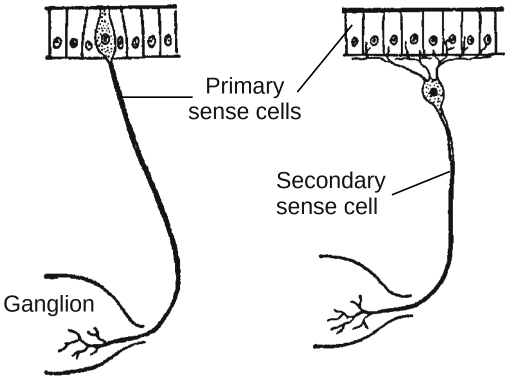

FIGURE 11.14. The differences between primary and secondary sense cells. Primary sense cells directly receive the stimuli. Secondary sense cells of non-neural origin are linked to primary sense cells. From Snodgrass (1935). Reprinted with permission.

Sensilla

The basic unit of sensory reception in insects is the **sensillum**, which originates from the same ectodermal tissues that give rise to the rest of the nervous system. A sensillum differentiates from a **sensillum progenitor cell** derived from the embryonic ectoderm as it expresses **proneural genes** during development that determine the outcome of differentiation and the type of sensillum that results. Division of the sensillum progenitor cell gives rise to the components of the mature sensillum, including the **sensory neurons**, a **tormogen cell**, which creates the socket; a **trichogen cell**, which creates the shaft of the hair; and a **thecogen cell**, which produces the sheath component of the sensillum that isolates the axons from one another and provides the neuron with ions and nutrients (Figure 11.15). The sensory neurons within the sensillum are bipolar, extending their dendrites into the cuticular portion and their axons to the central nervous system. Olfactory sensilla may contain the dendrites of one to five

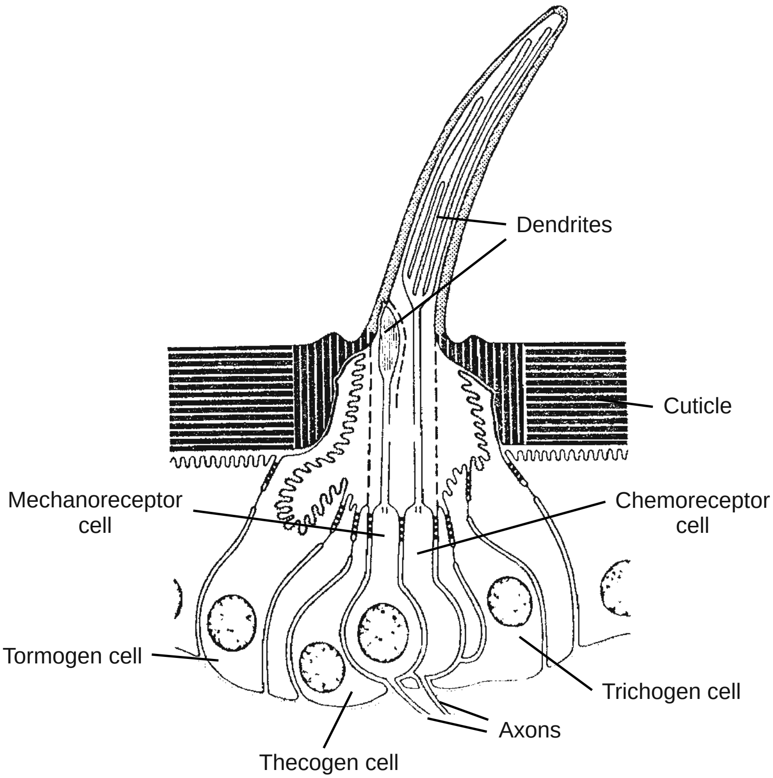

FIGURE 11.15. Basic structure of a cuticular sensillum. From Altner and Prillinger (1980). Reprinted with permission.

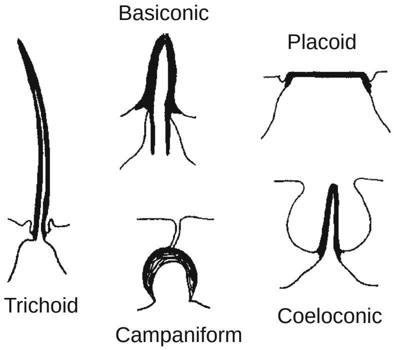

FIGURE 11.16. Examples of external morphologies of sensilla. From Zakaruk (1985). Reprinted with permission.

olfactory receptor neurons. Although most sensilla are associated with the outer surface, some, such as stretch receptors, are suspended between two points on internal organs or internal parts of the integument.

Sensilla may be structurally categorized by their external morphology (Figure 11.16), but structure alone is usually not sufficient to determine function. Although gross sensillar morphologies may be similar, the fine structure of a particular sensillum may vary considerably depending on the sensory modality involved. Nevertheless, a structural classification scheme of insect sensilla is most common. **Trichoid sensilla** have long hairs and one to three unbranched dendrites covered by a thick cuticle. **Basiconic sensilla** are shorter with one to three branched dendrites and a thicker cuticle. **Placoid sensilla** are platelike, with two to 50 branched dendrites. **Coeloconic sensilla** have a peg-like structure and are often located within a cuticular pit, innervated by three to five unbranched dendrites. Another way of classifying these sensilla is based on a functional classification according to the stimuli the receptors are believed to respond to. This classification system is used here.

Chemoreceptors

Two general types of chemoreceptors are capable of responding to chemical energy in the environment. **Gustatory chemoreceptors**, which function in what we commonly refer to as taste, respond to substances in solution at relatively high concentrations and a relatively close range. They are concentrated mostly on the mouthparts, legs, and ovipositor. The axons from these taste chemoreceptors connect with interneurons in the ganglia of the segment on which they appear. **Olfactory chemoreceptors** on the antennae and on the palps of the mouthparts mediate what is considered as smell, responding to

substances traveling in air in relatively low concentrations and originating at greater distances. The axons from these olfactory receptors generally terminate in the deutocerebrum. Each antenna of *Drosophila* adults typically contains about 1200 olfactory receptor neurons.

Both taste and olfactory chemoreceptor sensilla often have the same outward appearance of a hairlike process or a small peg, but they differ in the number of pores that are present on the shaft. For taste receptors, the soma of hairlike trichoid sensilla lie beneath the hair, and the dendrites extend into the shaft where they reach a single pore at the tip (Figure 11.17). Taste receptors on the legs may additionally have mechanoreceptive dendrites associated with them. Internal subcuticular taste receptors can also be found within the digestive tract (Figure 11.18).

Sensilla involved in taste perception contain **gustatory receptor neurons** (**GRNs**) that are specialized for either sugar, water, or concentrations of salt. Bitter-tasting deterrents inhibit the sugar receptor and stimulate the GRN for high salt concentrations. The gustatory receptors of *Drosophila* are members of the *Gr* gene family of G-protein-coupled receptors with 70 putative members, and in the mosquito *Anopheles gambiae*, 76 *AgGr* have been identified. These gustatory receptors are expressed in gustatory organs and specifically localized in the GRNs. The gene *Gr5a* encodes a receptor for the sugar trehalose.

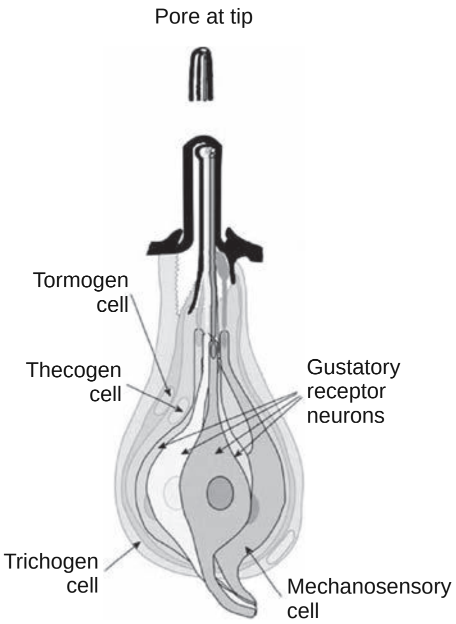

FIGURE 11.17. A gustatory sensillum. From Ishimoto and Tanimura (2004). Reprinted with permission.

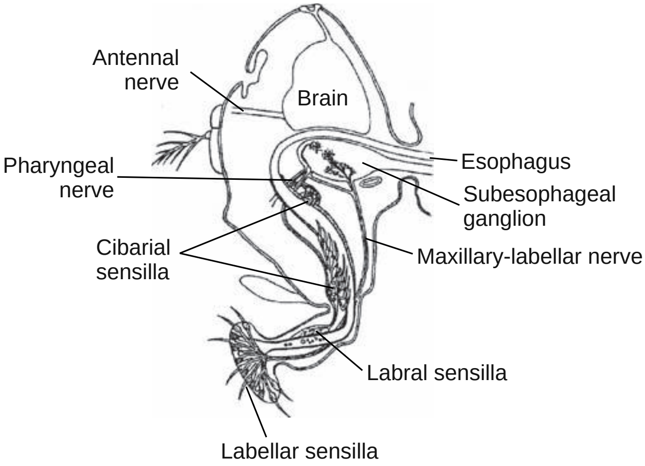

FIGURE 11.18. Internal subcuticular sensilla within the digestive tract of a fly. From Mitchell et al. (1999). Reprinted with permission.

Axons from most of the gustatory sensilla in the head run directly to the subesophageal ganglion without synapsing. Those in the tarsi project into the compound thoracic-abdominal ganglion. In contrast to olfactory neurons that express a single odor receptor, gustatory neurons may express several different taste receptors. They are also involved in other aspects of taste. The *Gr* gene *Gr68a*, expressed in taste sensilla on the front legs of male *Drosophila*, may be a receptor for nonvolatile contact pheromones and play a role in courtship behavior.

Olfactory chemoreceptor neurons (ORNs) are located within basiconic sensilla, which are blunt hairs or short cones, or hairlike trichoid sensilla on the antennae and maxillary palps (Figure 11.19). Olfactory sensilla differ from taste sensilla in that they have numerous pores along their shafts and lumens that contain one to five ORNs. Each antenna of the *Drosophila* adult bears approximately 1200 ORNs, and each maxillary palp has about 120. Sixty odor receptor genes (*Or*) code for a diverse family of Or proteins that are borne on the ORNs and determine their response characteristics. *Drosophila Or* genes are expressed in subsets of 3 to 50 ORNs, with those ORNs that express the same olfactory receptor extending their axons to the antennal lobes of the deutocerebrum. From there, second-order olfactory neurons send this information to the mushroom bodies, and integration of these pathways occurs by third-order neurons within the calyx region of the mushroom bodies.

The dendritic branches of the ORNs are bathed in **sensillum lymph**, a fluid high in potassium (Figure 11.20). The high potassium in the lymph, along with mucopolysaccharides in solution, binds water and regulates its overall hydration.

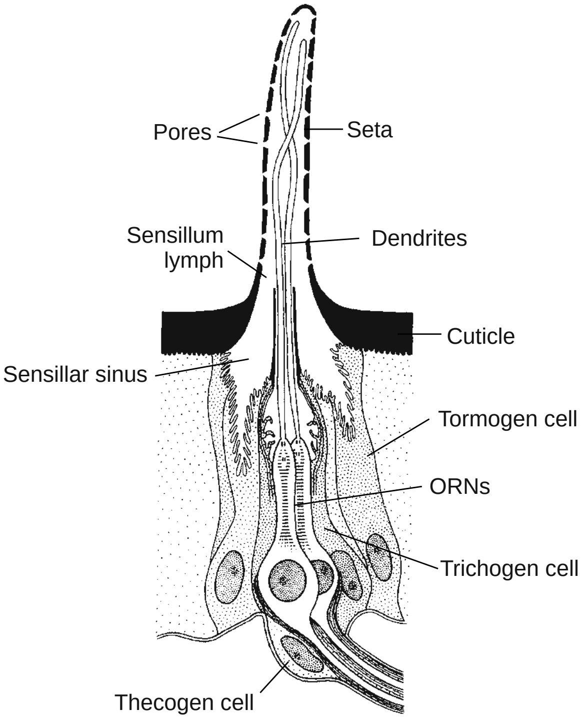

FIGURE 11.19. A trichoid olfactory sensillum. From Zacharuk (1985). Reprinted with permission.

The sensillum lymph also contains **odorant binding proteins** (OBPs) that are important in odorant transport and deactivation. The proteins are produced by the sensillar support cells and are secreted into the sensillum lymph. The OBPs that are contained within the lymph may provide another filter that can distinguish odor molecules. They are largely responsible for the specificity and sensitivity of insect olfaction, enhancing the rate at which odor molecules are captured and partitioning these often hydrophobic molecules in the aqueous system that surrounds the dendrite. Three classes of odorant binding proteins have been identified in the antennae of adult Lepidoptera: two classes of **general odorant binding proteins** that are consistent with the distribution of general odorant receptors and a third class of **pheromone binding proteins** that predominate in the antennae of male moths that have a large number of sensilla that respond to the female sex pheromone. There may be different odorant binding proteins for recognizing different odorants. *Drosophila* has more than 50 OBPs, with those expressed in pheromone-responsive sensilla considered to be pheromone-binding proteins. In male *Antheraea polyphemus* moths, about 70% of

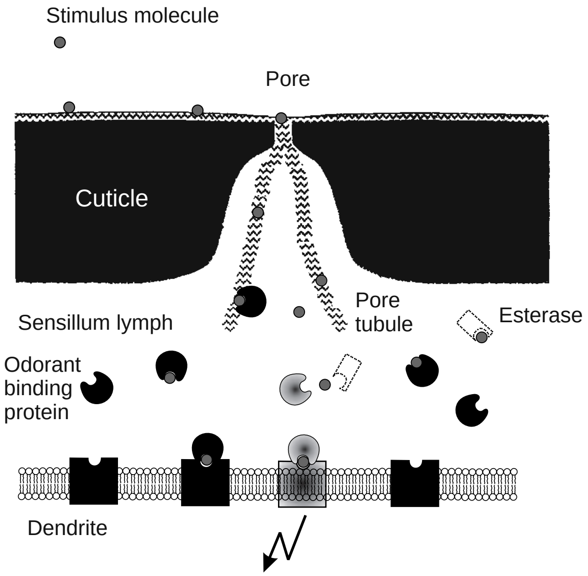

FIGURE 11.20. Model for the stimulation of a dendrite by a stimulus molecule. The molecule strikes the cuticle and diffuses across it to the pore tubule. It moves through the tubule into the sensillum lymph, where an odorant binding protein takes up the stimulus molecule and delivers it to the receptor on the dendritic membrane. This causes the excitation of the dendrite. Once released into the lymph, the stimulus molecule is degraded by esterases and the binding protein is recycled. From Steinbrecht (1997). Reprinted with permission.

the more than 60,000 sensilla present on the antennae are responsive to the female sex pheromone.

Almost all organisms create gradients of carbon dioxide that could provide meaningful information to recipients, and sensory structures for the detection of CO_2 are present in many insects. These CO_2 receptors tend to be located on the palps, found in lepidopteran larvae and adults (Figure 11.21) and in adult male and female mosquitoes. Basiconic sensilla on the maxillary palps of female *Aedes aegypti* mosquitoes respond to low concentrations of CO_2 in the range of 150 to 300 ppm and can detect increments as small as 50 ppm. Antennal sensilla that respond to CO_2 have also been identified in hymenopterans and *Stomoxys* stable flies.

Sensory Transduction

Sensory transduction refers to the ways that environmental energy is changed into electrical energy in the nervous system. Several distinct steps in this process have been identified for olfactory chemoreception (Figure 11.22).

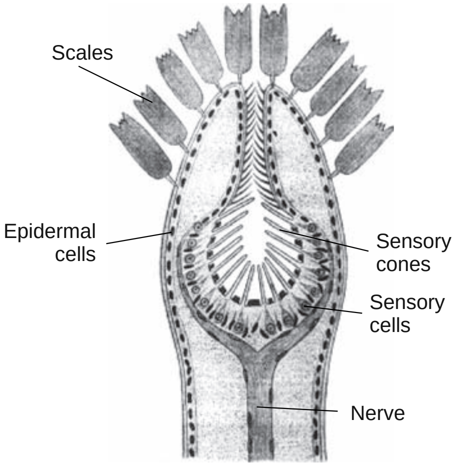

FIGURE 11.21. A carbon dioxide receptor. From Stange and Stowe (1999). Reprinted with permission.

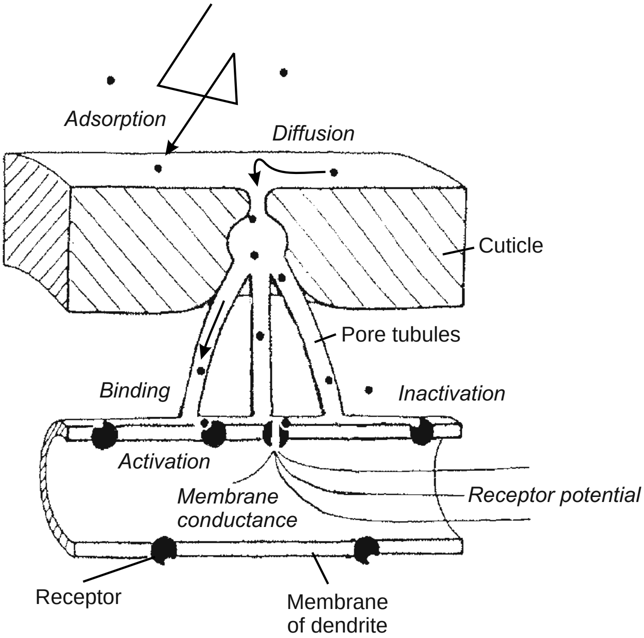

FIGURE 11.22. Mechanism of sensory transduction. From Kaissling (1974). Reprinted with permission.

The mechanism of transporting molecules from the outside to the surface of the dendrite can be efficient enough to allow a single odor molecule adsorbed on the cuticular surface to ultimately cause an action potential to be generated. In the first, **adsorption**, the odor molecule is adsorbed from the air onto the cuticular wall. The molecule then **diffuses** through the cuticle to the pores and **pore tubules** (Figure 11.23) in the shaft of the sensillum cuticle that lead into the lumen of the sensillum and direct the odor molecules to receptor sites on the dendrite through the sensillum lymph. **Binding** involves the specific binding of the odor molecules to receptor sites, followed by their conformational change and **activation**. The activated G protein-coupled receptor induces a change in **membrane conductance** that leads to the generation of an action potential. Once activated, the phosphorylated receptor binds to nonvisual **arrestins** that inhibit any subsequent receptor interactions and ultimately promote its internalization. Lastly, **inactivation** of the odor molecule must occur to make room for the next molecule. After the dendrite is stimulated, it must be rapidly inactivated to prevent its continuous stimulation and to allow the subsequent binding of another odor molecule to occur. An insect flying within an odor plume must make informed assessments about the concentrations of odorants in the plume and respond promptly and accordingly. **Esterases** within the sensillum lymph as well as on the surface of the cuticle quickly inactivate the odorant molecules to make room for subsequent stimuli. Those esterases that are present on the cuticle may prevent the entire surface of the insect from becoming a pheromone source for other insects when pheromones bind to it. The binding proteins that are present in the sensillum lymph may also be involved in the degradation process.

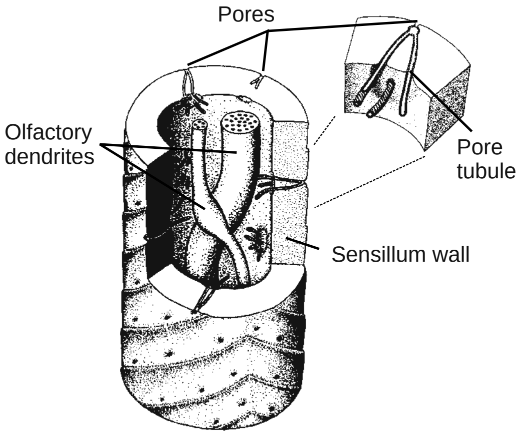

FIGURE 11.23. The pores and pore tubules within the shaft of an olfactory sensillum. From Keil (1999). Reprinted with permission.

Typically, thousands of receptors are present, many of which may show varying sensitivity to a given stimulus. To decipher the chemicals present in the environment, the brain must discern which of the receptors has been activated by an odor molecule and the degree of activation. The recognition of a variety of environmental molecules thus requires a large number of receptor proteins. The binding of a specific odorant requires a fairly specific receptor on the neuron, and with thousands of receptor neurons and an inestimable number of odorants, it would be difficult to account for the variety of these receptor proteins based simply on the 14,000 genes in the *Drosophila* genome. One possible explanation for the diversity of immune recognition proteins in the hemolymph may also explain this diversity of neuron receptors.

Two alleles of the *Drosophila* gene *Dscam* have been proposed to account for the specificity of neuron receptors, as they are capable of forming more than 38,000 different mRNA isoforms by way of alternative splicing. The **Dscam** cell surface proteins are associated with developing neurons and are required for axon guidance and dendrite patterning during development. The isoforms may give neurons their identities and mediate their spatial mapping interactions during the wiring process to form the functional neuronal network. Dscam (Down syndrome cell adhesion molecule) was first identified in humans as a member of the immunoglobulin superfamily class of receptors and mapped to the same chromosome that is associated with Down syndrome.

The brain may receive information from sensory receptors in two general ways. It makes use of those sensilla that specifically react to a narrow range of compounds, such as pheromones. When such a specialist receptor is stimulated in this **labeled line**, the brain "knows" that the particular compound must be present because it alone can cause the stimulation and the behavior is directed accordingly (Figure 11.24A). However, although the receptor variety is certainly impressive, it would be impossible for the sensory system to accommodate every possible stimulus molecule with its own dendritic receptor.

Other receptors have a much broader response spectrum with differing sensitivities, and rather than binding to a few specific receptors, a particular molecule may activate a wider range of more generalist receptors to greater or lesser degrees. The resulting code of neural activity from a field of generalist receptors is known as **across-fiber patterning** (Figure 11.24B). This allows a large number of different stimuli to create different patterns that can be coded by the central nervous system without requiring large numbers of specific receptors for each molecule. The receptor code may also change with stimulus concentration, allowing the many receptors involved to functionally perceive a different odor.

Both strategies of sensory transduction may occur. In several phytophagous insects, including the Colorado potato beetle and the locust, the quality of host plants is determined by a large number of contact chemoreceptors on the palps, without any single receptor being responsible for acceptance or rejection of the food. In other insects, such as the cabbage white butterfly, maxillary taste recep-

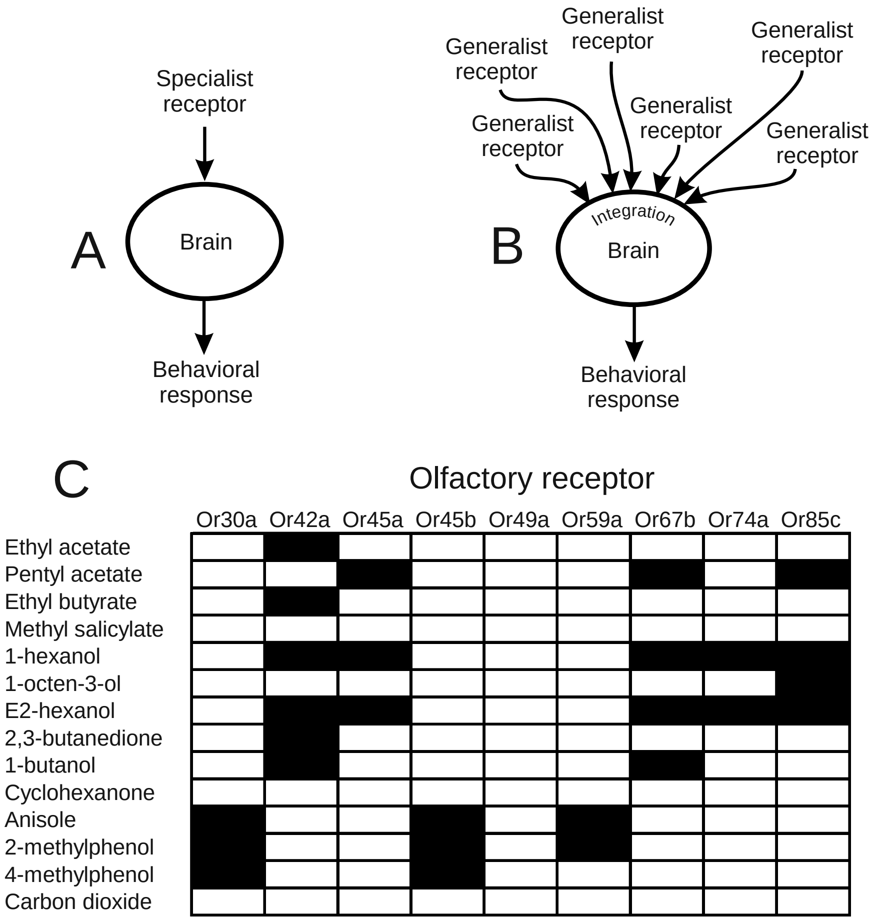

FIGURE 11.24. Production of a behavioral response from a labeled line (A) or an across fiber pattern (B), indicated by a filled box. In the labeled line, a specific receptor sends a specific message to the brain. In across fiber patterning, the overall pattern from a number of generalist receptors is evaluated by the brain. (C). The odorant receptors that respond to specific stimulus molecules, producing a pattern to the central nervous system. A filled box indicates a response of the receptor. From Kreher et al. (2005). Reprinted with permission.

tors are specific for glucosinolates, and the stimulation of these receptors evokes feeding behavior. Similarly, feeding deterrents may act via specific labeled lines or by disrupting the normal across-fiber patterns that the insect requires for feeding.

The responses of sensilla can be measured electrophysiologically. The electrical potential change between the base of the antenna and its tip can be measured when an odor molecule is passed over it and the receptors are activated. This change in electrical potential can be monitored as an **electroantennogram**,

which measures the summated receptor potentials in the whole antenna (Figure 11.25). **Single-cell recording**, in which an electrode is implanted at the base of a single sensillum and the signals from the single receptors are measured, provides a more precise determination of individual sensillar responses (Figure 11.26). This technique is able to determine the sensitivity and specificity of ORNs within individual sensilla. Both methods can provide preliminary information for screening potential attractants and deterrents, but neither can substitute for determining the behavioral responses of intact insects. The electrophysiological techniques fail to account for the effects of physiological state and nervous integration on the activation of behavior.

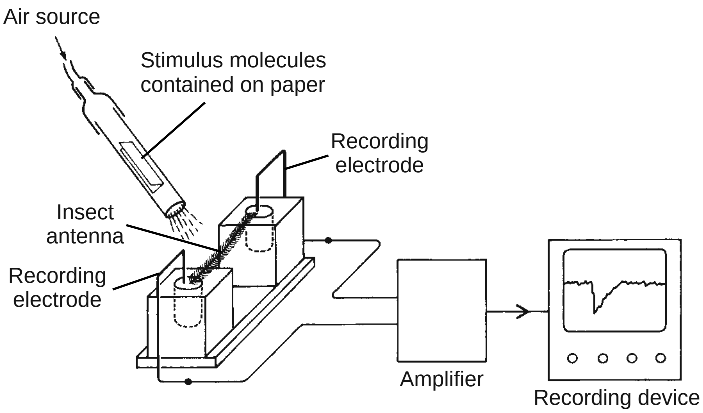

FIGURE 11.25. An example of an electroantennogram. The receptors on the whole excised antenna respond to the stimulus blown across it. The responses of all the sensory cells are amplified and displayed. From Gullan and Cranston (2000). Reprinted with permission.

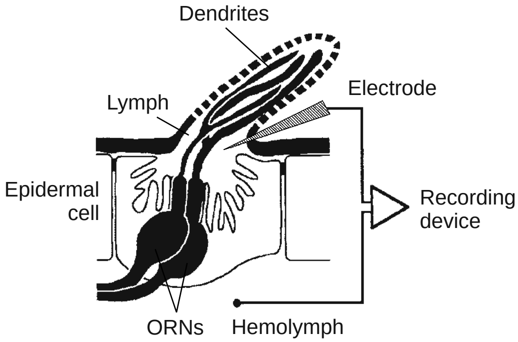

FIGURE 11.26. Single-cell recording from an olfactory sensillum. From de Bruyne et al. (1999). Reprinted with permission.

Thermoreceptors and Hygroreceptors

The sensitivities to temperature and humidity are often found in the same antennal receptors. Combinations of cold, moist air, and dry air receptors or cold and warm receptors are most common. Thermo/hygroreceptors are located at the top of the antennal club of some butterflies in the form of basiconic sensilla, typically small pegs located within cuticular pits (Figure 11.27). They can measure ambient temperature, and humidity may hydrate the cuticular peg causing it to deform and also function like a typical mechanoreceptor. Sensory neurons in the head and body wall of *Drosophila* larvae increase their firing rates when cooled and decrease them when warmed. Butterflies that bask with open wings to absorb heat from the sun bear thermoreceptors along the anal wing veins to detect temperatures that might be dangerously high and adjust their wings accordingly.

Mechanoreceptors

Mechanoreceptors sense mechanical energy in the environment, such as pressure, gravity, vibration, and the internal forces generated by muscles, as they distort

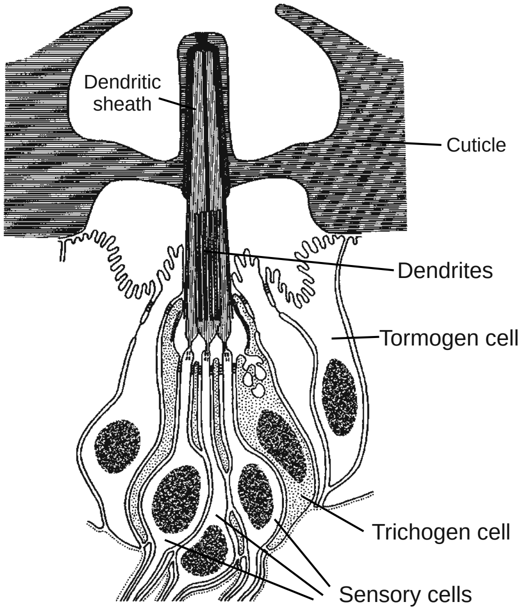

FIGURE 11.27. A sensillum that responds to humidity. From Altner and Loftus (1985). Reprinted with permission.

the body. The rigid exoskeleton is an ideal platform with which to detect these vibration stimuli and transfer them to sensory receptors. The various types of mechanoreceptors include cuticular structures, chordotonal organs, and stretch receptors.

The cuticular structures consist mainly of the hairlike trichoid sensilla that respond to tactile stimulation. These setae resemble the chemoreceptor sensilla but have no pores and are innervated by a single neuron. Some chemoreceptors may contain an additional mechanoreceptor at the base that operates independently of the chemoreceptor neurons. The tormogen, or socket cell, secretes a joint membrane that contains the elastic protein resilin to provide flexibility in its movement. Displacement of the seta deforms the tubular body of the dendrite and initiates a receptor potential that in turn triggers an action potential in the axon (Figure 11.28). Some trichoid sensilla display a **phasic response** that generates impulses only when deflected but not when constantly deformed. Other, usually blunter, sensilla show a **tonic response** that is strong when initially deformed and steady but reduced under constant deformation.

Campaniform sensilla are dome-shaped structures, usually located near joints or other structures such as the halteres, that are subject to distortion and cuticular stress (Figure 11.29). The **scolopale**, a cuticular cap, covers a single neuron underneath that is stimulated by its deformation when compressive forces are generated in the adjacent cuticle. The scolopale consists of longitudinally arranged microtubules that are surrounded by actin filaments. The campaniform

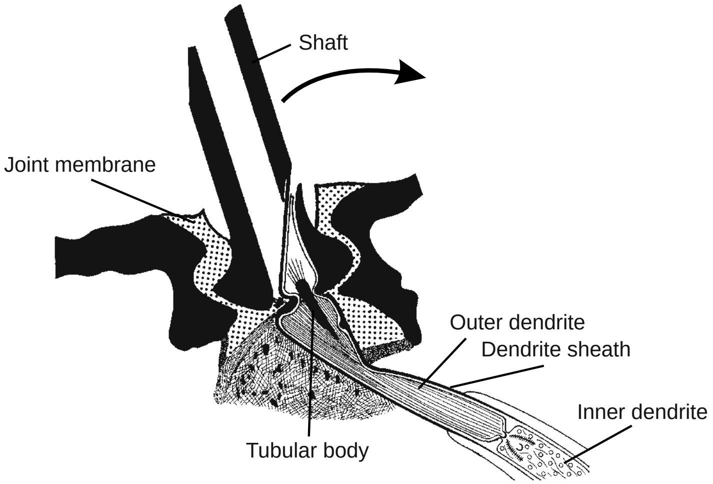

FIGURE 11.28. A mechanoreceptor that is activated by movement of its seta in the direction of the arrow. The deformation of the subcuticular tubular body initiates the receptor potential. From Barth, F.G., R. Blickhan. 1984. *Biology of the integument*, vol. 1, pp. 554–582. Copyright Springer–Verlag GmbH & Co. KG.

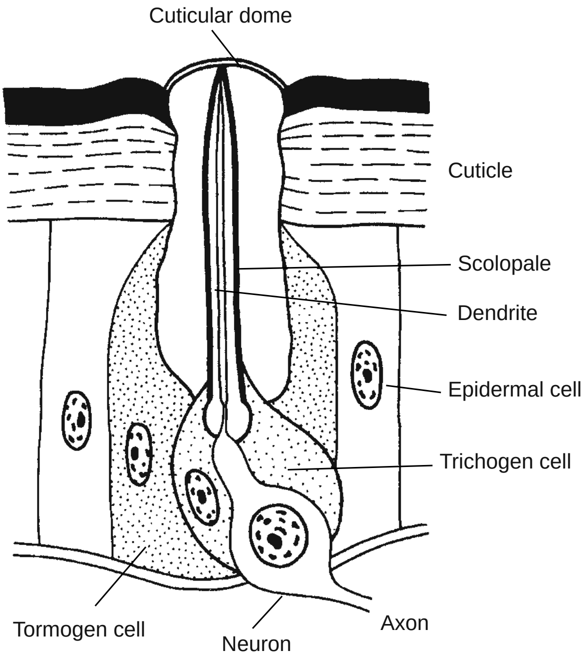

FIGURE 11.29. A campaniform sensillum. Sheer forces within the cuticle deform the cuticular dome. The resulting movement of the scolopale generates a receptor potential in the dendrite. From Chapman (1991). Reprinted with permission.

sensilla on the cerci of the cricket initiate a kicking response by the legs when they are deformed by movement of the cercal shaft.

Chordotonal organs consist of subcutaneous groupings of special sensilla known as **scolopidia**, which serve as mechanical transducers that detect cuticular displacements. An individual scolopidium is composed of four cell types that include one or more bipolar sensory cells, a glial cell, an attachment cell, and a scolopale (Figure 11.30). It is usually attached to the cuticle at both ends and can thus measure the degree of cuticular distortion. Integumental movement causes the scolopale to lengthen, compressing the dendrite and triggering a receptor potential by affecting the opening of ion channels. Chordotonal organs are internal sense organs and are never associated with external cuticular processes. They are often used as **proprioreceptors** to measure relative body position in response to cuticular deformation by bending, gravity, airborne vibrations, or air movement. Many hemipterans use plants as a transmission medium for vibratory signals that are perceived primarily through the chordotonal organs in the femur and tibia.

Chordotonal organs are also involved in insect hearing. **Johnston's organ** is found in the antennal pedicel of many insect orders and responds to minute

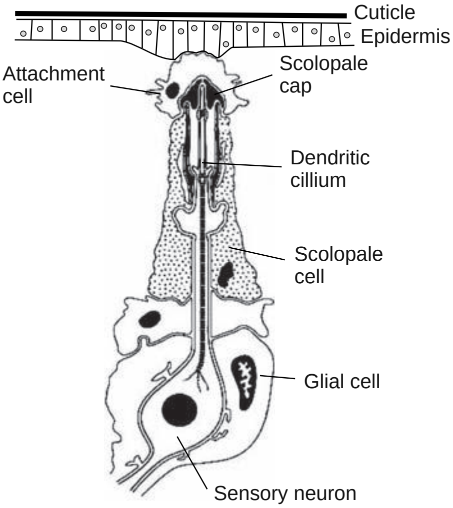

FIGURE 11.30. A subcuticular chordotonal organ. Attached to the cuticle at both ends, these sensilla can measure the degree of cuticular distortion. From Yack (2004). Reprinted with permission.

displacements of the flagellum. In many dipterans, a large dorsal bristle, the **arista**, receives vibrations that are relayed to the antennal shaft and transmitted to the groups of scolopidia below, allowing the insect to detect vibrations produced by individuals of the opposite sex and to receive feedback from air currents that deform the antennae during flight. In nematocerous Diptera, Johnston's organ functions much like a joystick, with the long flagellum receiving vibrations that are transferred to the ring of scolopidia within the pedicel (Figure 11.31). Male mosquitoes use their Johnston's organ, containing more than 7000 associated scolopidia, to identify conspecific females based on their flight tone and wing-beat frequency. Johnston's organ on the antenna of *Corethrella* mosquitoes allows them to identify and locate tree frogs by their calls.

Subgenual organs are chordotonal organs located in the tibia of many insects, attached at one end to the cuticle and at the other to a trachea, but never associated with the leg joint (Figure 11.32). They respond to vibrations of the leg, perhaps as currents of hemolymph are created by the movements. The organ is employed in a variety of ways, from the most sensitive known insect vibration detector to an auditory organ. It may help the insect to avoid predators or to respond to intraspecific signals. The subgenual organ in the tibia of the cockroach, *Periplaneta*, responds best to sounds of 1.8 kHz and to a displacement of as little as 2 nm. Many species of neuropterans are able to use subgenual organs to detect substrate-borne vibrations for species recognition and mating (Figure 11.33).

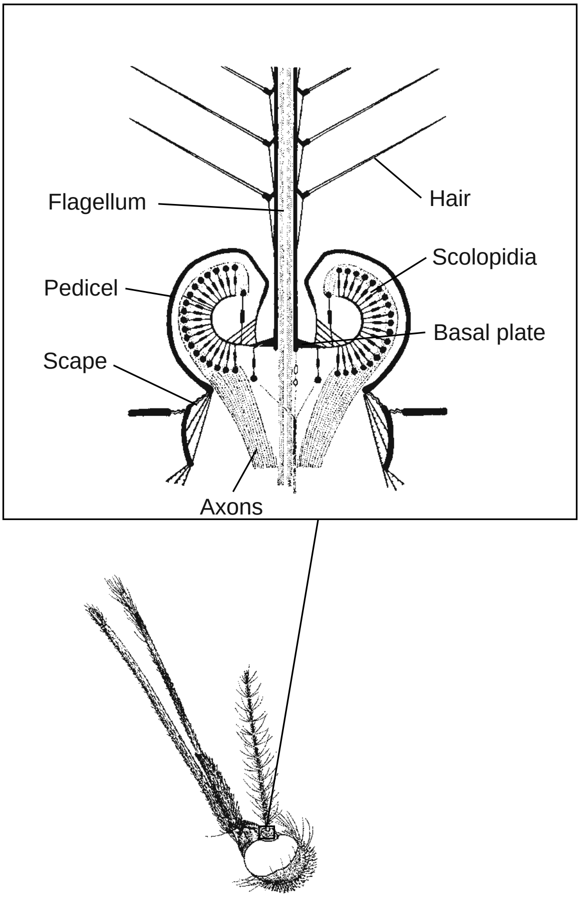

FIGURE 11.31. The antenna of a mosquito. Within the box identified here is a cross section of Johnston's organ at the base of the antenna. The basal plate deflects the scolopidia when it is displaced by movements of the flagellum.

Tympanal organs are chordotonal structures that detect sound vibrations, and with an increasing association with the tracheal system, they may have evolved from subgenual organs (Figure 11.34). The tympanal organ consists of a thin, drumlike piece of cuticle called a **tympanum** with a chordotonal organ with from one to several scolopidia attached on the inside that can monitor its movement. Sounds cause the tympanal membrane to vibrate, and the scolopidia detects the vibrations (Figure 11.35). The tympanum enables insects to respond to a broad range of frequencies from 2 kHz to as high as 140 kHz, with a maximum sensitivity in the range of 30 to 60 kHz. They are often present in a

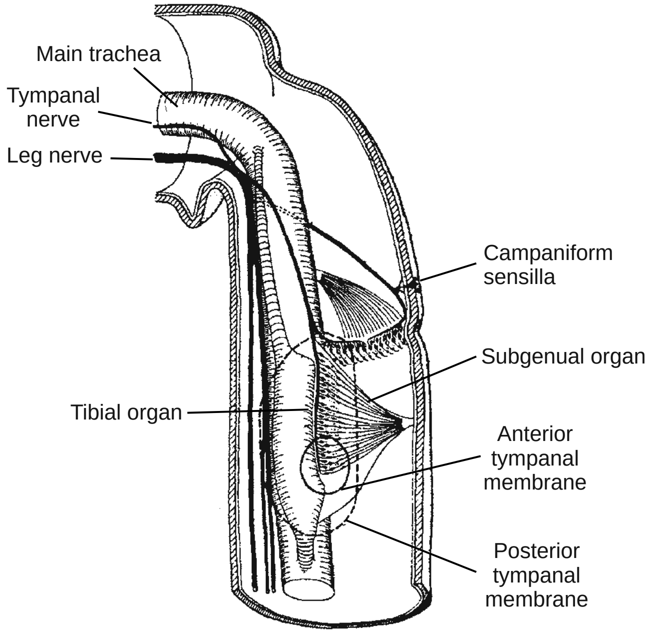

FIGURE 11.32. A subgenual organ within the tibia of an insect leg. One end is attached to the cuticle, and the other end is attached to the trachea. From Field and Matheson (1998). Reprinted with permission.

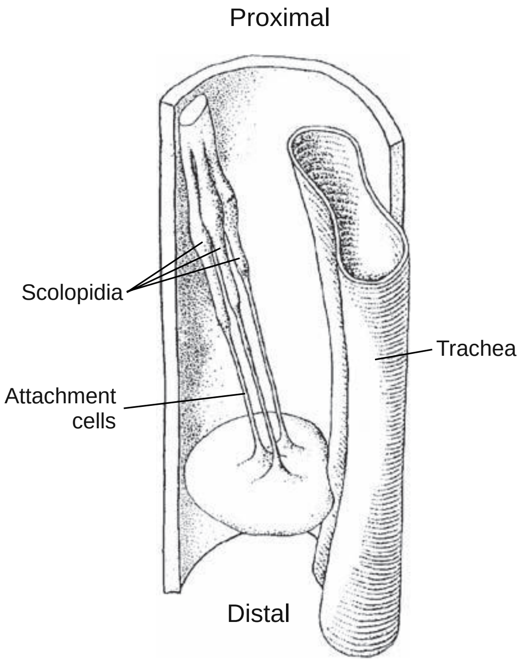

FIGURE 11.33. A subgenual organ that detects substrate vibrations in the leg of a green lacewing. From Devetak and Pabst (1994). Reprinted with permission.

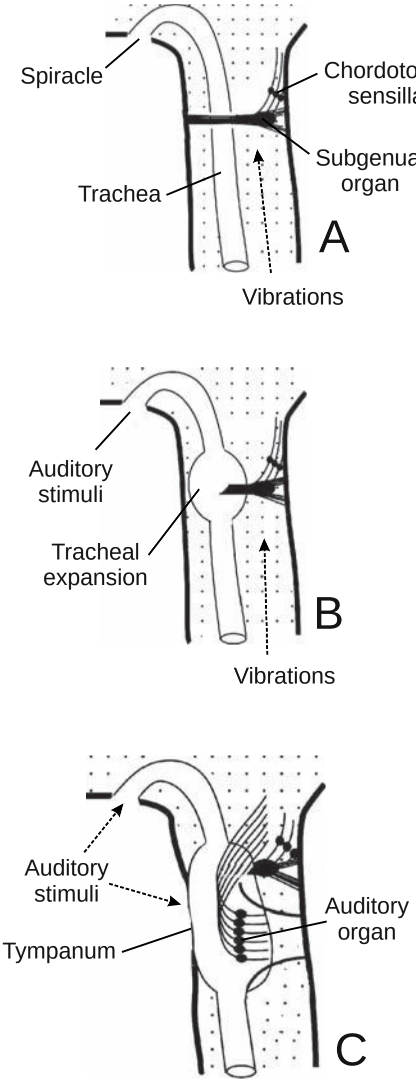

FIGURE 11.34. Beginning with a subgenual organ (A), the possible evolution of a tympanum (C) resulting from an increasing association with the tracheal system.

widely separated, paired configuration to allow the detection of directionality. By comparing the intensity at the two tympana, the insect can determine the direction of a source of sound. In crickets, tympanal organs are present on the fore tibia with their membranes backed against a large tracheal tube. In some grasshoppers, the organ is located on either side of the first abdominal segment.

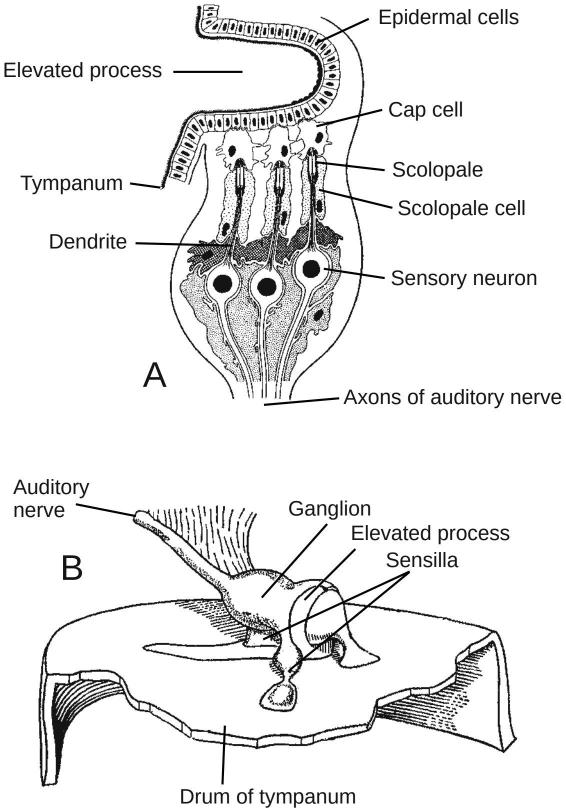

FIGURE 11.35. A. A cross section of the sensillum underlying the tympanum. B. The inner surface of a tympanal organ. From Gray (1960). Reprinted with permission.

Praying mantids have a single ear on the ventral midline of the thorax located within a longitudinal groove. Green lacewings bear small tympana on the swollen radial wing veins, differing from other insect tympana in having a fluid-filled cavity above the tympanic membrane (Figure 11.36).

The interaction between moths and bats has been characterized as an evolutionary arms race. The night-flying moths are able to perceive the echolocation systems of preying bats and execute evasive behaviors in response, whereas the bats have altered their calls to overcome the moths' defenses. An elegant system with only two scolopidia coupled to each abdominal tympanal organ at the junction with the thorax mediates their responses. There are two sensory cells, A1 and A2, associated with each tympanum, and each of the cells has a differing sensitivity (Figure 11.37). In noctuids, the A1 cell is more sensitive to sound than the A2 cell. The cell with the greater sensitivity responds to the more

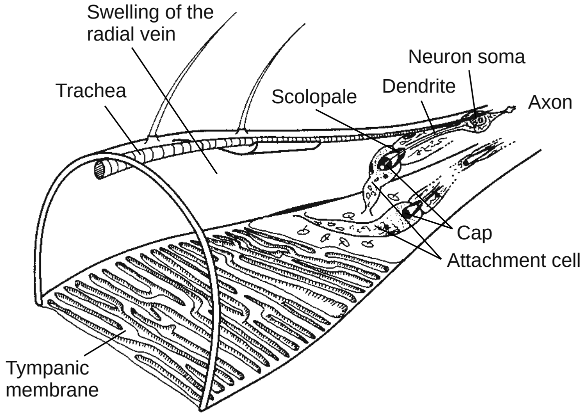

FIGURE 11.36. The tympanum on the wing of a green lacewing adult. From Miller (1970). Reprinted with permission.

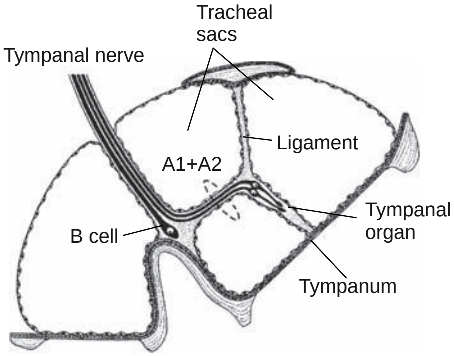

FIGURE 11.37. The tympanal organ of moths, consisting of a tympanum innervated by two sensory cells (A1 and A2) with differing sensitivities. From Yeager (1999). Reprinted with permission.

distant cries (>5 m) and causes a movement away from the sound, whereas the less sensitive cell is triggered when the bat is nearby (<5 m) and causes the moth to immediately engage in an evasive dive. Another neuron, called the B cell, has some degree of response but is considered to be nonauditory. Although bats can use higher frequency calls to reduce their detectability, these have a decreased range.

Stretch receptors are multipolar neurons that are found throughout the internal organs. They consist largely of dendritic endings embedded at several points in muscle, connective tissue, or within the basement membrane that surrounds an organ (Figure 11.38). They provide feedback for proprioreception

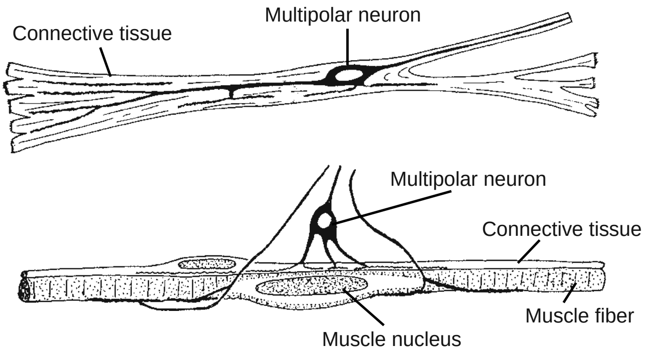

FIGURE 11.38. Two stretch receptors. (*Top*) A multipolar neuron associated with the connective tissue of a cockroach. (*Bottom*) A multipolar neuron associated with the muscle fiber and a strand of connective tissue in a moth. The dendrites of the multipolar neuron extend into the fiber tract. From Osborne (1969). Reprinted with permission.

when the structure that is monitored changes in shape. The foregut is often innervated by multipolar neurons that can respond to gut movement and distention. Within the abdomen, dorsal longitudinal stretch receptors may be present in each segment that extend to the intersegmental regions.

Infrared Receptors

Infrared irradiation can be detected by paired thoracic pit organs in the species of buprestid beetles that breed only in trees that have been recently killed by fire. The beetles respond to the infrared emitted from forest fires in the wavelength range of 2 to 4 μm using dome-shaped sensilla located in paired pits near the mesothoracic coxa that are exposed during flight (Figure 11.39). Each pit organ contains 50 to 100 sensilla with lenslike cuticular structures that change in volume with exposure to infrared radiation and deform the dendrite of the underlying mechanoreceptor. These sensilla are activated specifically by the infrared wavelengths from burning forests, and other sensilla on the antennae respond to olfactory cues from phenolic compounds present in the smoke from these recent fires.

VISUAL RECEPTORS

Highly mobile animals like insects require visual cues to maneuver and identify relevant objects in complex environments. The sensitivity to visual stimuli is possible through a number of different receptors in insects that offer a range of

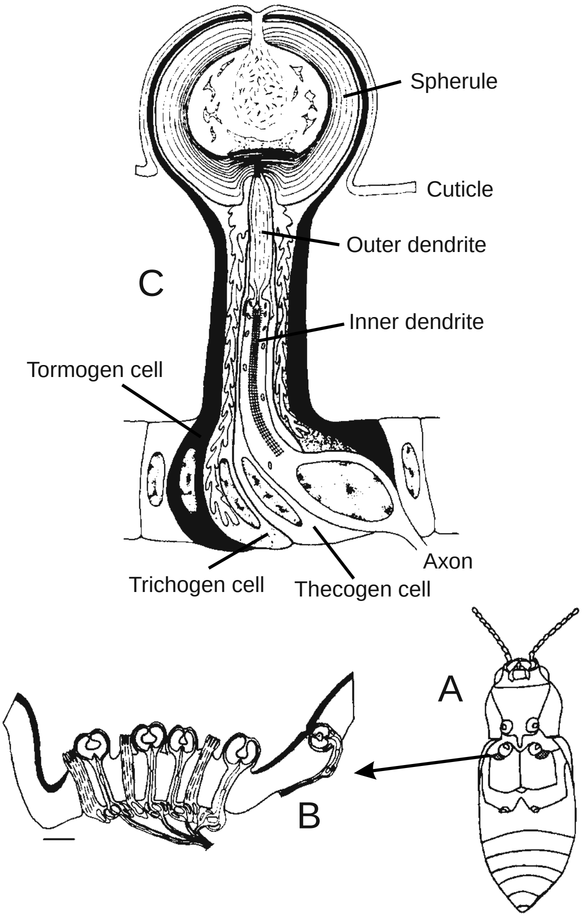

FIGURE 11.39. A receptor (C) from a buprestid beetle that is located in a cuticular pit (A, B) that responds to infrared emanations from forest fires. From Vondran et al. (1995). Reprinted with permission.

sensitivities and resolutions to match ecological requirements. Perception of light is not enough, as it must be accompanied by the ability to compare the light coming from several directions and provide the animal with spatial vision. Simple photoreceptors that respond to light do not help the organism use the light to orient itself unless they also provide this spatial vision.

Many organisms have evolved eyes that enable them to visualize the environment, and it has been proposed that as many as 65 independent evolutionary events were responsible for the current diversity of animal photoreceptors. However, molecular evidence suggests that similar pathways for eye development

exist in both vertebrates and insects and that the evolution of eyes among all animals may actually be monophyletic. The homeotic master gene ***Pax 6*** regulates eye development in both humans and mice. A homolog of *Pax 6* in *Drosophila*, the gene *eyeless*, ultimately controls eye development there also. When the mouse *Pax 6* gene is transgenically expressed in *Drosophila*, it directs the cascade of fly developmental genes to produce a compound eye ectopically in the area of its expression. With a common master gene and the evolutionary intercalation of a series of other gene products, one could easily imagine the evolution of eyes in all metazoans from a common ancestor. However, this monophyletic hypothesis has been criticized for its failure to account for the presence of different transduction mechanisms in the ciliated rod and cone photoreceptors of vertebrates and the rhabdomeric receptors of invertebrates. The identification of both types in the marine ragworm, the last common ancestor of insects and vertebrates, suggests that animal eyes have indeed evolved at least twice and perhaps even more often.

Insect photoreceptors can be classified as simple eyes or compound eyes. Simple eyes are the **dorsal ocelli** found in many winged adults and the **stemata** of larval holometabolous insects. **Compound eyes**, present in adult holometabola and larval and adult hemimetabola, have multiple optical systems that provide much greater resolution and are more capable of motion detection.

Dorsal Ocelli

Of the receptors responding to light in the visual spectrum, **dorsal ocelli**, or simple eyes, are the least complex of the visual structures. They often appear clustered in a triangular pattern on the head between the compound eyes in the winged adults of most orders and in the larvae of hemimetabola, but they may also occur singly. A typical ocellus consists of a convex transparent corneal lens with an aggregation of hundreds of light-sensitive **retinula cells** below (Figure 11.40). A region of each of the retinula cells known as the **rhabdom** contains the visual pigment **rhodopsin** that absorbs light and initiates the receptor potentials. A layer of cells underneath that contain urate crystals or closely packed tracheae may function as a reflecting **tapetum**. The axons from these retinula cells synapse with a small number of interneurons so that fewer axons enter the brain than the number of receptor cells present, effectively limiting its resolution even further. In flies, the three ocelli are connected to the protocerebrum by a single ocellar nerve. In locusts, 600 to 800 retinula cells synapse with only six interneurons. The ocelli thus appear to be poorly designed for image perception and may be able only to scan the horizon for light intensity to provide general information for navigation during flight. They serve as rotation detectors in conjunction with the compound eyes to maintain the insect's rotation in space. Sensitive to ultraviolet light, they appear to be horizon detectors that interact

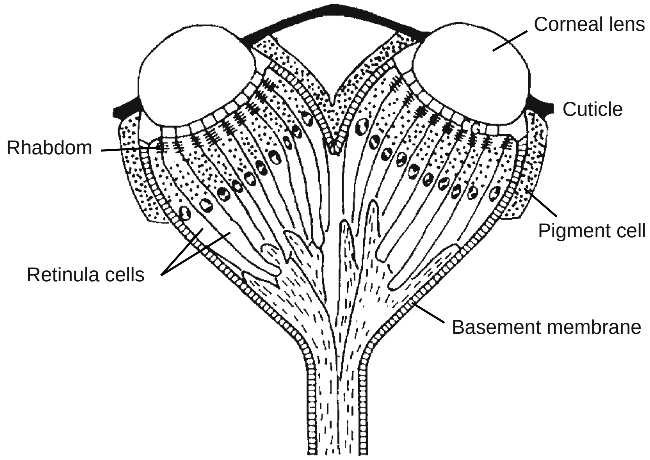

FIGURE 11.40. The generalized structure of a pair of ocelli. From Toh and Tateda (1991). Reprinted with permission.

with the processing of stimuli from the compound eyes to facilitate locomotion that is based on the perception of visual images.

Lateral Ocelli

Lateral ocelli, or **stemmata**, are also relatively simple eyes that are the only photoreceptors present in the larvae of holometabolous insects and generally fail to persist through metamorphosis to the adult. Despite the name, their structures are more similar to the compound eyes than to the dorsal ocelli, but they fail to meet the image quality that is possible with compound eyes. The number of receptors associated with stemmata is usually too low to allow the formation of anything but a coarse mosaic of the environment. The larval holometabolous insects that bear these stemmata generally crawl and have no need for a visual system with the higher performance of the compound eyes in adults, where flight and mate identification require better image processing. Typically, a stemma bears a cuticular **corneal lens** above a **crystalline cone**, and these serve as the optical elements (Figure 11.41). As in the dorsal ocelli, a portion of the plasma membrane of the retinula cells is specialized as a **rhabdomere** to contain a large number of microvilli that contain the visual pigment. The increase in surface area that the rhabdomere provides allows those neurons to pack abundant visual pigments into each cell. These may contain multiple photopigment systems, suggesting that color discrimination is possible. Two or more rhodopsins that

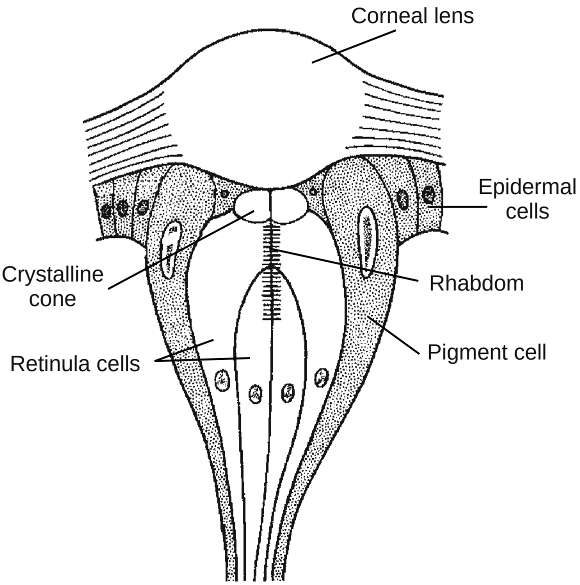

FIGURE 11.41. A lateral ocellus, or stemma. From Gullan and Cranston (2000). Reprinted with permission.

are tuned to different regions of the visual spectrum can allow the insect to discriminate visual stimuli on the basis of their wavelengths. The stemmata may also be sensitive to polarized light.

Depending on the species, there may be from as few as three to more than 5000 retinula cells below that are grouped around a central rhabdom consisting of the rhabdomeres of the retinula cell, and several of these rhabdoms may be present within a single stemma. The light from the lenses focuses on one or more of the rhabdoms. Stemmata may be present in insects, either in groups or singly, and provide a coarse mosaic of the environment but with far better resolution than the dorsal ocelli. The largest stemmata, with exceptional resolution, are those of the tiger beetle larvae, with six units on each side of the head and more than 6000 retinal receptors. Larvae of the swallowtail butterfly have six stemmata on each side of the head, each with seven retinula cells that contribute to a single fused rhabdomere. The retinula cells within a stemma have sensitivities to green, blue, or ultraviolet light.

Larval stemmata are not necessarily eliminated during metamorphosis but can migrate to the posterior surface of the optic lobe of the developing adult. In adult *Drosophila*, they are present at the posterior margin of the compound eye known as **Hofbauer-Buchner eyelets**, and their homologs may be present in several other insects. The eyelets consist of pigmented organs with numerous

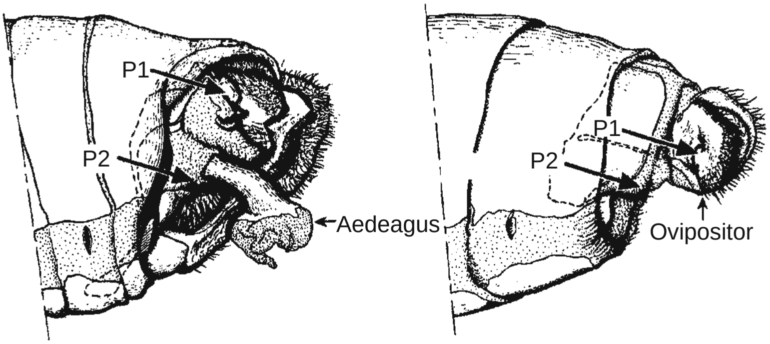

FIGURE 11.42. The genital photoreceptors in male (*left*) and female (*right*) Lepidoptera. From Arikawa (2001). Reprinted with permission.

microvilli that are arranged as rhabdomeres and contain optical pigments and clock proteins. These **extraretinal photoreceptors** innervate a portion of the brain thought to house the circadian pacemaker and are suspected to play a role in circadian perception in the adult and the entrainment of locomotor behavior.

Genital photoreceptors have been identified in many adult butterflies, developing during the pupal stage. These receptors provide little in the way of resolution and are thought to be involved in oviposition behavior, informing the female that the ovipositor is extended far enough to successfully lay eggs. They also play a role in copulation in the male, providing information that the vagina of the female is aligned properly for penile insertion. Two pairs of genital receptors, P1 and P2 (Figure 11.42), mediate these behaviors in both sexes. Their structures resemble that of a **phaosome**, a primitive photoreceptor first identified from the epidermis of earthworms. The presence of photoreceptor pigments within the genital photoreceptors has yet to be confirmed.

Compound Eyes

Compound eyes are the primary visual receptors of adult insects and larval hemimetabola. They are paired structures located on either side of the head capsule, each consisting of multiple optical systems in contrast to the single optical systems of ocelli and stemmata. They contain a few to several thousand groups of **ommatidia**, the individual self-contained optical units, each with its own lens system and underlying light receptors (Figure 11.43). Dragonflies may have as many as 30,000 ommatidia that occupy most of the head, a worker honeybee has about 5500, *Periplaneta* cockroaches about 2000, the *Drosophila* has 800, and subterranean insects may have fewer than 20, if any are present at all.

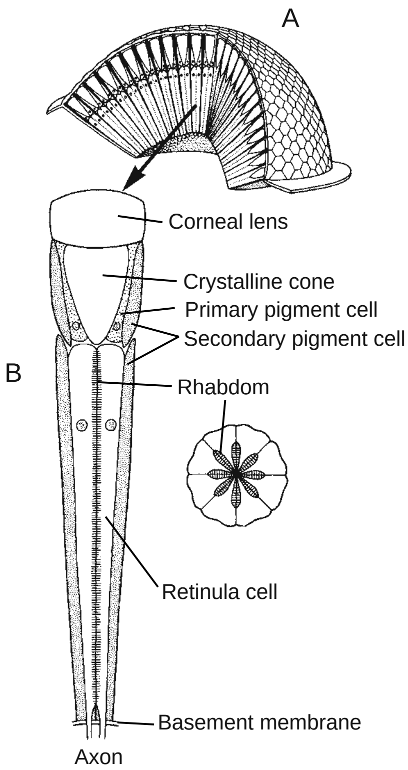

FIGURE 11.43. A. A section of the compound eye showing the position of the ommatidia. B. A cross section of a single ommatidium. From Gullan and Cranston (2000). Reprinted with permission.

In the primitive proturans and diplurans, compound eyes are completely absent. The ommatidia of some insects may be of different sizes in different areas of the compound eyes. For example, the aquatic beetle, *Gyrinus*, has a dorsal pair of eyes that looks above the water and a ventral pair of eyes that looks below into the water. Not all the facets that face the environment may respond similarly; different ommatidia in the compound eyes of honeybees process visual information differently, with shape detection and pattern and color discrimination processed best in the ventral part of the frontal visual field. Each lens forms a small image of the field of view, and the central nervous system patches these views together.

Eyes are receptors that evaluate the three-dimensional world by projecting images on a two-dimensional field, and the nature of that field in insects differs from that in vertebrates. In vertebrates, the receptor surface is concave (Figure 11.44A), allowing a single lens in front of the retina to produce an image. In

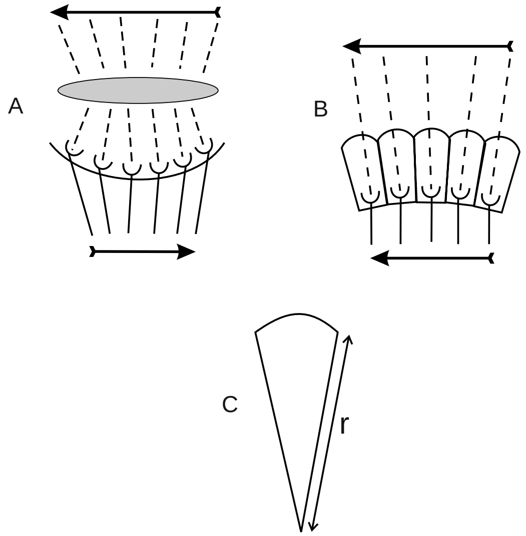

FIGURE 11.44. A. The lens of vertebrates projects a reversed image on the concave receptor surface. B. The lens system of the insect compound eye projects an unreversed image on a convex receptor surface. C. The resolution of the ommatidium is dependent on the radius of its curvature (r). From Goldsmith (1990). Reprinted with permission.

arthropods, the receptor surface is convex, and an image is formed only when the individual ommatidia allow a narrow cone of light to enter that is perpendicular to the receptor surface (Figure 11.44B). Even though each ommatidial lens focuses an inverted image on the receptor surface, the overall image that the brain receives is an erect one that is constructed from the individual fields of view of all ommatidia. This design has implications for image resolution, as it is primarily dependent on the radius of the eye's curvature (Figure 11.44C). Each ommatidial field of view is about 1° across. A larger radius provides an increased resolution but brings with it larger facets, and there are limits to the size of the eye that can be accommodated on an insect's head. A doubling of resolution would require the eye to increase four times in size. A compromise in some insects is the development of a **foveal region** where the radius of curvature of some of the ommatidia is large and visual acuity in the region is greater.

The compound eyes provide a panoramic view of the world with a large field of vision. Even ants, with a relatively small number of facets on either side of the head, can perceive almost the entire visual field above and below the horizon except for a blind area of about 10% of the total field that lies below the thorax and abdomen. In the design of the insect eye, visual acuity is sacrificed for a panoramic view of the world. Members of the Diopsidae, a family of

dipterans, bear their compound eyes on stalks. The 2500 ommatidia in each eye provide a significant increase in the stereoscopic vision compared to flies without eye stalks.

Compound eyes may be placed in one of two categories: the **apposition eyes** of most day-active insects or the **superposition eyes** of nocturnal insects that sacrifice resolution for increases in sensitivity. There is considerable variation in design within each of these categories.

Apposition Compound Eyes

The apposition eye design is most common in insects that are active during the day and probably represents the ancestral condition. The ommatidia are optically isolated from each other by the sheath of screening pigment that surrounds them. Because light strikes the light-sensitive rhabdom only if it enters through a single lens at a narrow angle from above, each rhabdom has its own optical system, much like having individual cameras pointed outward. Because the images are processed in parallel, the design allows for fast motion detection and image recognition. The resolution of the apposition eye is a function of the angle between adjacent ommatidia, the lens diameter, and the total number of the units that make up the eye. To approach the resolution of the human eye, a compound eye would require absurdly large components (Figure 11.45).

The optical portion of the apposition eye consists of a modified cuticle that forms a **corneal lens** of about 10 to 25 μm in diameter (Figure 11.43). The lens is produced by two modified epidermal cells, the **corneagen cells**, which move to the outer edge of the ommatidium later in development and produce the masking pigments. Bristles or conical protuberances may be present on or at the edge of the cornea to increase the visual capacity of the eye and reduce reflection to also provide camouflage. **Semper cells** produce a second lens, the **crystalline cone**, which is surrounded by pigment cells. The corneal lens and crystalline cone together focus the light on the optically active receptor of the ommatidium and are isolated from neighboring units by the pigment cells. Elongated retinula cells in groups of 8 to 12 are arranged around the longitudinal axis of the ommatidium. Their cell membranes along the ommatidial axis contain dense microvillar borders that constitute the rhabdomere of each cell. The rhabdomeres of the packed retinula cells are closely apressed to form the rhabdom, onto which the light is focused. As in the stemmata, they contain the visual pigment rhodopsin, which becomes activated when it absorbs light. Each ommatidium is approximately 200 to 300 μm in length and consists of about 30 cells.

One compromise in the design of the apposition eye is that image brightness is diminished because each facet only captures a small amount of light. Some Diptera and Hemiptera possess a variant of the apposition eye that collects light

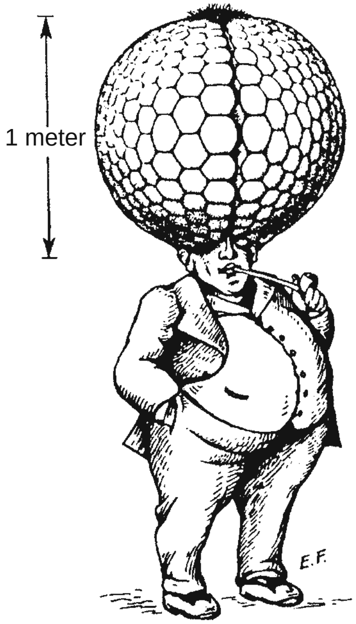

FIGURE 11.45. The approximate size of a compound eye in humans if the same resolution were to be obtained as for a lens eye. From Kirschfield (1976). Reprinted with permission.

more efficiently, called a **neural superposition eye**. Its optical arrangement is the same as for an apposition eye, but the receptors are organized in a different way. Rather than having a typical rhabdom that consists of the fused individual rhabdomeres of the retinula cells, the rhabdomeres are unfused and receive and transmit separate images that are reconstructed neurally so that the rhabdomeres of different ommatidia that view the same point converge on the same synapse, making them superposition by neural configuration rather than optical design (Figure 11.46). Like optical superposition, discussed next, spatial information from one point in the visual field is collected by a number of ommatidia but is superimposed neurally to form the final image. Each point in the field of view may stimulate seven rhabdomeres in adjacent ommatidia, and the neural wiring allows each axon peering into the same field to make the same connections with interneurons, making the signal seven times stronger than otherwise possible. This allows the insect to see fairly well at dusk and dawn when competitors and predators that lack this advantage are inactive.

A **pseudopupil** may be visible in many apposition eyes when they are viewed by an observer. This small dark spot moves across the field with the changing external viewpoint. The pseudopupil represents the pigmented region of the ommatidium that absorbs light and thus shares a common line of sight between

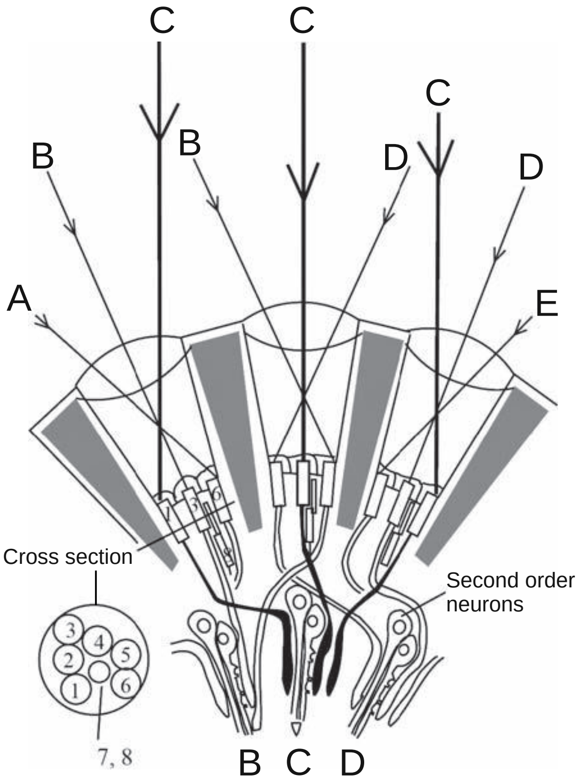

FIGURE 11.46. A neural superposition eye. The points of light arriving at the same angle stimulate the unfused rhabdomeres that send their messages to the same synapse, superimposing the image neurally. From Horridge (2005). Reprinted with permission.

the insect and the observer. It looks dark because it absorbs light from the direction of the observer. Studies utilizing the pseudopupil have allowed the internal structure of the ommatidium to be examined without destructive histological sectioning.

Superposition Eyes and Adaptations for Dim Light

The visual system of insects is well adapted for perceiving objects in bright light, and the apposition eyes of most flying insects allow them to be active during the daylight hours. However, performance of the eye is poor under reduced illumination. The ability to remain active under dim lighting can be a significant advantage, allowing the insect to avoid day-active predators and exploit resources

such as nocturnally flowering plants that are not present during the day. Some originally diurnal-active species that modified their behavior and evolved a nocturnal habit retained their apposition eyes but developed an increased light sensitivity. For example, some nocturnal wasps have 25% more facets than their diurnal counterparts to maximize their light-gathering ability. The rhabdom cross-sectional area of the nocturnal halictid bee, *Megalopta genalis*, is 16 times larger than that of the day-foraging honeybee, and the diameter of the facets almost twice as large, resulting in a 30-fold increase in their physical light-gathering ability. The additional presence of branching interneurons in the optic ganglion has been proposed as a mechanism that allows a neural summation of the visual signal from several ommatidia (Figure 11.47).

The rhabdom of apposition compound eyes normally extends from the basement membrane of the ommatidium up to the crystalline cone, with screening pigments within each surrounding pigment cell that prevent light from the other ommatidia from striking the rhabdom and the pigments of apposition eyes but that do not move within the cells. In contrast, the rhabdom of the **superposition** eye is separated from the crystalline cone by a larger distance, called the **clear zone** (Figure 11.48A), and facets are not isolated to the same degree. In darkness, the pigment granules in both primary and secondary pigment cells move upward and reduce the isolation between neighboring ommatidia so images from several facets are projected onto a common receptor. The granules are concentrated between the crystalline cones, taking less than an hour for complete adaptation. In the clear zone of dark-adapted insects, no pigment is present to block the passage of light across it. A dark-adapted superposition eye can spread the light from as many as 30 ommatidia, increasing the sensitivity but

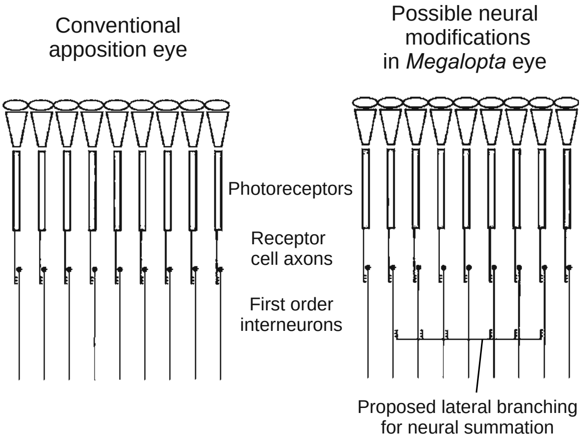

FIGURE 11.47. Neural summation from several ommatidia in the *Megaloptera* eye as an adaptation for perceiving low levels of light. From Warrant et al. (2004). Reprinted with permission.

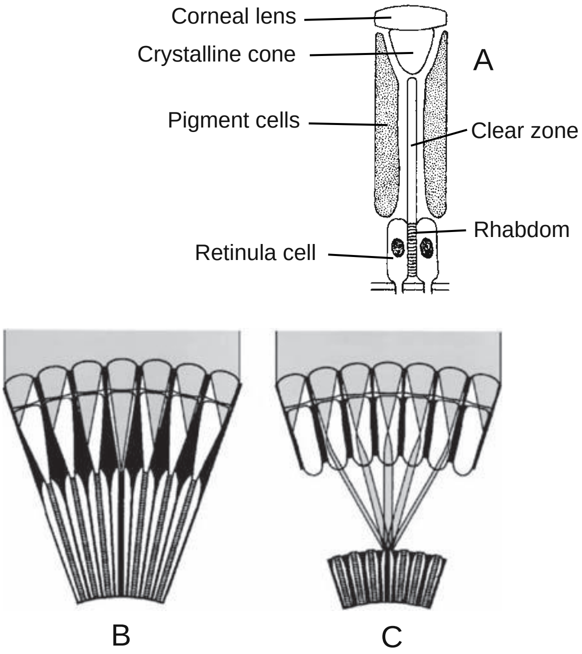

FIGURE 11.48. The structure of the superposition eye. A. The rhabdom is separated from the optical portion of the eye by a clear zone. B. The mechanism of light stimulation in the apposition eye. Light from neighboring ommatidia is blocked by screening pigments. C. Light from neighboring ommatidia of the superposition eye stimulates the same rhabdom. From Warrant et al. (2004). Reprinted with permission.

decreasing the resolution and increasing the blurring at image interfaces (Figure 11.48C). The reduced ability of some *Drosophila* mutants that lack eye pigments to resolve images is apparent in behavioral tests because the light they receive is spread over a wider visual field. Back in the light, the pigments migrate to their former positions and conventional apposition vision is utilized. Ultraviolet light impinging on the region beneath the crystalline cones and on the rhabdom itself causes the migration of pigments.

A unique superposition eye that possesses both high spatial resolution and high sensitivity has been identified in the day-active hummingbird hawk moth, *Macroglossum stellatarum*. The eye lacks conventional ommatidia, but has up to four rhabdoms under each facet, and variation in facet packing produces gradients of resolution and sensitivity in different areas of the eye. Unencumbered by the typical ommatidial matrix, the rhabdoms have formed acute zones that result in gradients with large superposition apertures that improve the performance of the eye for fixating flower entrances when they may be moving rapidly from the wind.

VISUAL PIGMENTS

Light-sensitive pigments are present in nearly all branches of organisms that respond to light, from bacteria to humans. The visual systems that have been examined in living organisms share a structurally similar optical receptor molecule, suggesting that the evolution of visual receptors is an ancient event. **Opsins** are proteins with seven-transmembrane domains belonging to the family of G-protein-coupled receptors that bind intracellular G-proteins when they are activated. The *Drosophila* compound eye expresses seven opsin genes that each has a unique spectral property and is associated with specific ommatidia. The absorption spectrum of the visual pigment determines the spectral sensitivity of the photoreceptor that expresses it. The helical opsin domains form a binding pocket that can accommodate vitamin A–derived retinoids, and together they form the visual pigment, **rhodopsin**. Insects use either the retinoids **11-*cis* retinal** (A1) or **11-*cis* 3-hydroxyretinal** (A3). A1 is considered to be the ancestral retinoid present in most insects, and A3 is believed to have been first adopted by insects about 65 million years ago. Some insect species use both retinoids. Rhodopsins are specifically localized in the microvillar membranes of the rhabdomeres and can be categorized according to their sensitivity to blue, green, or ultraviolet (UV) light. Each sensory neuron expresses only one type of sensory receptor.

Various amino acid side groups shift the spectral sensitivity of the molecule; a single amino acid polymorphism is believed to be responsible for ultraviolet vision in invertebrates. Different opsins have characteristic absorption peaks that determine which wavelengths of light may be absorbed, and the presence of these different opsins in different photoreceptor cells determine the degree of color vision possible. Opsins that are sensitive to ultraviolet wavelengths below 400 nm are found not only in the rhabdoms of ocelli and compound eyes of bees but also in the optic and antennal lobes of the brain and may provide a way for UV light to play a role in the regulation of circadian rhythmicity. Extraretinal nonvisual photoreceptors containing UV opsins have been identified in the **laminar organs** located in the optic lobes.

The absorption of light by the visual pigments causes an isomerization of 11-*cis* retinal to the *trans* configuration (Figure 11.49), activating the rhodopsin portion of the pigment to **metarhodopsin** (Figure 11.50). When the active metarhodopsin that results catalyzes G-protein activation, the activated G-protein activates phospholipase C (PLC). PLC catalyzes the breakdown of the membrane phospholipid phosphatidyl 4,5-biphosphate (PIP_2) into inositol trisphosphate (IP_3) and diacylglycerol (DAG), which causes **transient receptor potential** (TRP) channels to open and an influx of Na^+ and Ca^{2+} that causes a depolarizing receptor potential to be generated (Figure 11.51). The *Drosophila* photoreceptors have the fastest G-protein signaling pathway known, able to respond 10 times more quickly than that of the rod in mammalian eyes. Unlike the visual cycle in

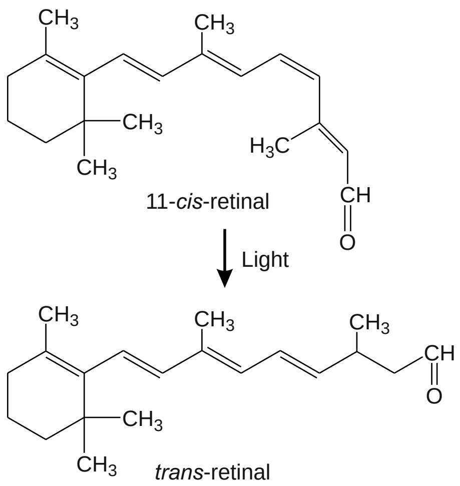

FIGURE 11.49. The two forms of retinal that combine with opsins to create rhodopsin. The 11-*cis* retinal is an unstable form that is converted to the *trans* form when it absorbs a photon of light.

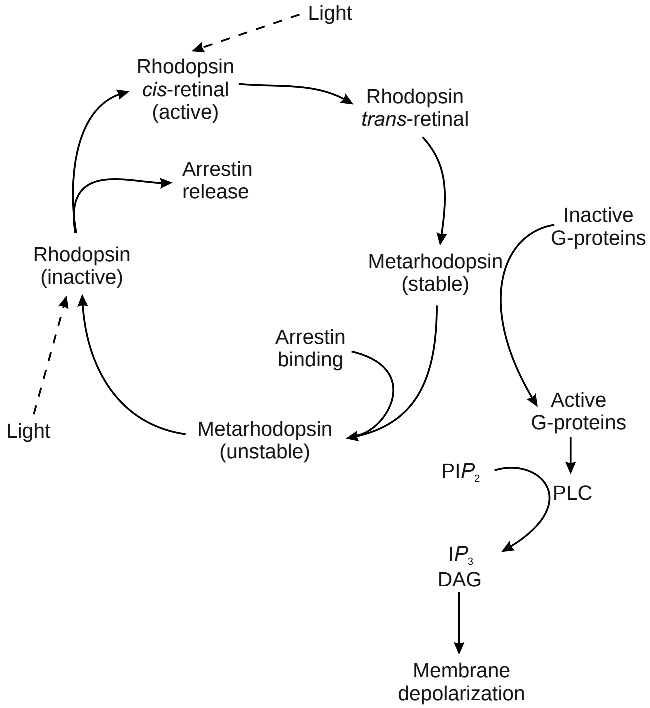

FIGURE 11.50. The biochemical pathway involved in the activation of rhodopsin and the resulting membrane depolarization. The unstable *cis*-retinal is converted to its *trans* form when it absorbs a photon of light. Its transformation to the stable form of metarhodopsin activates G-proteins that ultimately causes the membrane depolarization. The metarhodopsin loses its ability to activate the G-proteins after it is phosphorylated and binds with arrestin proteins to assume its unstable form. Light converts this metarhodopsin to an inactive rhodopsin that releases its arrestin and again becomes capable of responding to light.

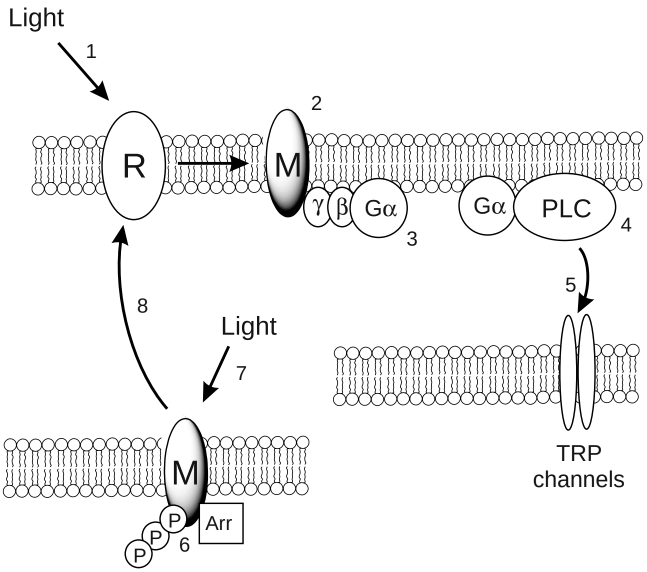

FIGURE 11.51. The transduction cascade on the *Drosophila* rhabdom membrane. (1) When a photon of light is absorbed by the rhodopsin (R) and mediates the isomerization to the *trans* form and (2) ultimately the stable metarhodopsin M, a G-protein cascade (3) is activated. This activates phospholipase C (PLC) (4) that ultimately activates transient receptor potential (TRP) channels (5) to allow the influx of sodium and calcium and generate a receptor potential. Phosphorylation and arrestin binding inactivates the metarhodopsin (6), but light (7) regenerates the rhodopsin once again (8).

vertebrates, where the absorption of light is only required for activation, the insect metarhodopsin is thermally stable and requires absorption by a second photon for regeneration of the active rhodopsin. Ambient light diffusing through the eye reconverts the metarhodopsin back to rhodopsin. As in vertebrates, the phosphorylated metarhodopsin is inactivated by arrestin binding, a factor also responsible for its thermal stability.

Drosophila has eight photoreceptor retinula cells in each ommatidium arranged in a trapezoid pattern that can be placed into three classes of spectral sensitivity. There are six outer photoreceptors that are sensitive to blue wavelengths of light, and the remaining two central neurons are sensitive to either blue-green light or ultraviolet wavelengths. Some ommatidia in regions of the compound eye may lack some of these pigments. The dorsal ommatidia of most insects lack green receptors; in the honeybee, these regions have only receptors for blue and UV light, suggesting they might be best adapted for detecting objects against the open sky or the sky itself. The spatial arrangements of the visual pigments can also modify their spectral sensitivity. When the rhabdoms are in a **fused** arrangement, the rhabdomeres that bear different pigments act as lateral filters for each

other. When the rhabdoms are **tiered**, distal receptors filter the light that reaches more proximal receptors. Many insects have a combination of both fused and tiered rhabdoms.

The rhabdomeres of *Drosophila* are open and function as independent light guides, whereas those of some bees, mosquitoes, and beetles are fused and share the same visual axis (Figure 11.52). Within the mosquitoes, the nocturnal *Anopheles gambiae* has a fused system that may contribute to image brightness but the rhabdomeres of the day-active *Toxorhynchites brevipalpis* are unfused. The gene *spacemaker* (*spam)* codes for a secreted protein that prevents the rhabdomeres from fusing to one another. Whereas *Tx. brevipalpis* expresses *spam*, *An. gambiae* does not. The spam protein thus partially accounts for the evolution and assembly of rhabdomere configuration.

Cryptochromes (Crys) are light-sensitive nuclear flavoproteins that regulate gene expression and have circadian clock functions in both invertebrates and vertebrates. Cry is found in neurons of the brain in *Drosophila*, where it functions as a blue light photoreceptor and interacts with clock transcription proteins to set the biochemical oscillator involved with circadian entrainment.

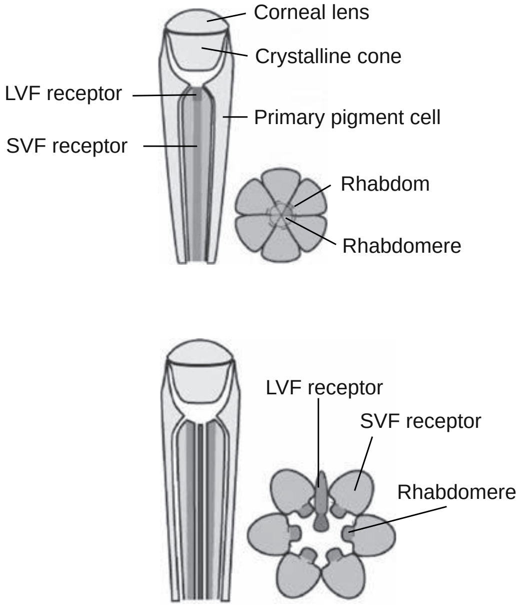

FIGURE 11.52. The fused rhabdomeres of bees and mosquitoes (*top*) and the unfused rhabdomeres of *Drosophila* (*bottom*). Long visual fibers (LVF) are the axons of photoreceptor cells that project through the first optic ganglion, and the short visual fibers (SVF) only project up to the first ganglion. The fused form is considered to be the ancestral form. From Osorio (2007). Reprinted with permission.

Perception of Polarized Light

Although the light originating from the sun is unpolarized and vibrates in all directions, the particles it encounters as it travels through the earth's atmosphere cause it to become polarized and vibrate in a specific direction. The degree of polarization changes with regard to the position of the sun and the orientation of the observer, making it possible to determine the sun's position even when clouds obscure it, as long as a portion of the sky is visible. Many insects can make use of this phenomenon for navigation by detecting polarized light. This ability is present in many social Hymenoptera such as bees, ants, and wasps that must orient to find food and return to their nests.

The ability to perceive polarized light lies largely within the orientation of the visual pigment within the rhabdomere. Some of the elongated rhabdomeres contain uniformly oriented rhodopsin within their microvilli (Figure 11.53), and the absorption of light is maximal when the light is polarized in the same direction as the pigment is oriented. If the receptors are moved as the insect rotates about its vertical axis, the output of the receptors is modulated as the microvilli become parallel to the light. Other receptors held at different angles record

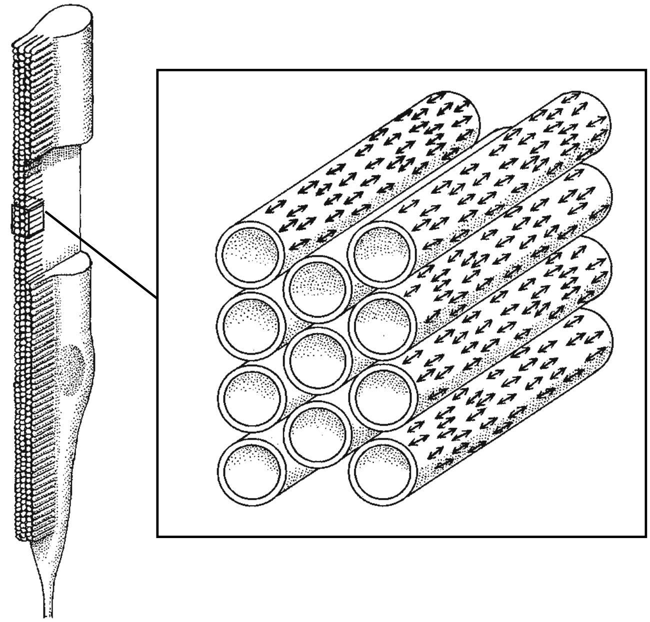

FIGURE 11.53. (*Left*) An elongated rhabdomere. (*Right*) The uniform orientation of rhodopsin within the microvilli of the rhabdomere that allows the reception of polarized light. From Wehner (1976). Reprinted with permission.

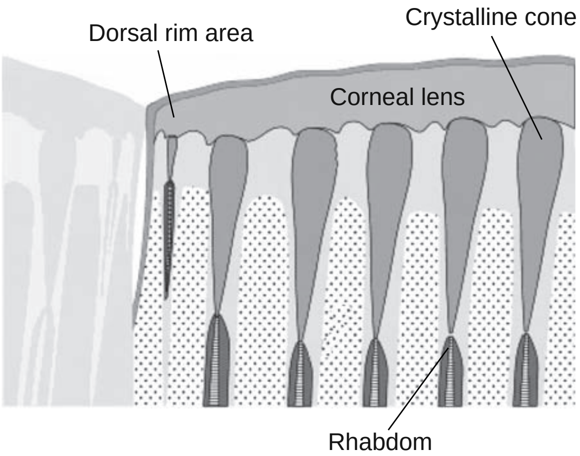

FIGURE 11.54. The specialized ommatidia in the dorsal rim area of the compound eye of some hymenopterans that are considered to be involved in the detection of polarized light. Conventional ommatidia are on the right. From Labhart and Meyer (1999). Reprinted with permission.

different responses to the rotation within the field of polarization. By scanning the sky, the insect can record the degree of polarization from the pattern registered in its receptors and can then later orient by matching the pattern in the sky with its recorded pattern in memory. Water can polarize reflected light, and aquatic insects can use their polarized light detection to identify the water surface.

In bees and ants that show polarization sensitivity, there is also a group of specialized ommatidia at the dorsal margins of the compound eyes believed to be involved in the detection of polarized light (Figure 11.54). The rhabdoms within this dorsal rim area are shorter with a larger cross-sectional area, and their microvilli, containing oriented pigments, are also oriented 90° to each other. Many other insects bear these dorsal rim area ommatidia but have not yet been studied for the ability to detect polarized light.

MAGNETIC SENSITIVITY

Some insects, including migrating monarch butterflies, honeybees, cockroaches, and ants, are sensitive to geomagnetic fields and may orient based on the field strength. Monarchs contain magnetic particles, thought to be magnetite (Fe_3O_4), that are synthesized in the thorax during metamorphosis to the adult. Although they become disoriented after being exposed to a brief magnetic pulse in the laboratory, there is little evidence that they use the earth's magnetic field for orientation. Bees use magnetic fields to supplement other information when attempting to locate foraging sites. Iron granules are deposited in the trophocytes

of the fat body, where expansion and contraction of the granules from magnetic fields may initiate a neural response in the workers. The foraging response is impaired if the insects are artificially treated with magnets attached to their abdomens. The migratory ant, *Pachycondyla marginata*, preys on termites and contains magnetic iron oxide particles in the antennae and abdomen that may explain its ability to orient parallel to magnetic fields. In many cases, the response to magnetism is supplemented by a response to light. The mealworm beetle, *Tenebrio molitor*, orients to magnetic fields only when light is additionally present.

Although the magnetic orientation behaviors have been documented for many insects, a convincing transduction mechanism is still lacking to explain how magnetic fields can affect the nervous system. In other animals, magnetic particles are coupled to stretch receptors or to photoreceptors whose light sensitivity is altered in the presence of magnetic fields.

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CHAPTER 12

Communication Systems

The preceding chapters have described the systems of communication that exist between the cells and tissues of individual multicellular organisms, but there is also communication between two or more whole organisms. Intraspecific and interspecific communications are absolutely essential for an organism's survival and reproduction. To reproduce, an individual must find and accurately identify the opposite sex so that it does not waste time and energy on mating attempts with stray objects in the environment that would fail to result in the production of offspring. Insects are frequently the prey of other animals, and there has been intense selective pressure for the evolution of defensive communications that minimize this loss. Also, because the competition for resources is most vigorous among the members of one's own species that occupy an identical ecological niche, communication with others is necessary to recognize this competition so that individuals might be identified and cooperated with, avoided, or possibly forced away from an area.

Many definitions of communication have been proposed, but none has been completely satisfactory to everyone. Animals produce many signals, not all of which are considered to be communications. Some definitions require that the interchange between two individuals must be in some sort of code whose structure has been forged through the process of natural selection in order for the

Characteristic	Type of Signal			
	Visual	Acoustical	Tactile	Chemical
Range	Medium	Long	Short	Long
Rate of signal change	Fast	Fast	Fast	Slow
Circumvention of obstacles	Poor	Good	Poor	Good
Energetic cost	Low	High	Low	Low

FIGURE 12.1. The characteristics of some signals used in insect communication. From Lewis (1984). Reprinted with permission.

signals that are produced to be classified as a communication. The reception and interpretation of this code — whether visual, auditory, or chemical — must be mutually beneficial by this criterion and result in the increased fitness of the participants. However, insect communication will be considered here in its broadest sense; if one individual gives off any signal that produces a change in the behavior of another individual, that signal will be considered to have been a communication. This chapter reviews some of the ways that insects produce these signals.

One way to classify the systems of communication is according to the receptors that are involved in receiving the stimuli. We can identify various chemical, tactile, acoustical, and visual signals as being the primary means of communication in insects, each with its own advantages and disadvantages (Figure 12.1). The reliance of a species on one signal over another is primarily a function of the ecological context in which the insect must function. For example, with the exception of those species that produce their own light, insects that are active at night are generally less dependent on visual cues for communication. Although signaling systems will be described individually, it must be emphasized that the expression of a behavior in natural situations often requires the involvement of signals using several sensory modalities. Tactile communication, for example, often elicits behavioral changes only in conjunction with other visual and chemical cues.

VISUAL COMMUNICATION

Insects use visual signals primarily to identify food and mates and to orient themselves in the environment. The compound eyes are most important in receiving the visual signals because they have the best resolution of the optical receptors present. Visual signals are often releasers of the highly ritualized and stereotyped insect behavioral sequences. Some insects have the surprising capacity to actually identify other individuals on the basis of their facial and abdominal characteristics. There is a dominance hierarchy in *Polistes* wasp colonies where queens and workers can visually recognize their nestmates as individuals and determine their unique status based on the yellow body markings.

Visual Tracking

Another important function of visual communication is the identification and tracking of food by using the visual signals from prey. Of the insects that communicate using vision for obtaining food, the visual system of the praying mantis may be the best understood. To capture prey, the praying mantis uses its two compound eyes to determine the distance of its prey on the background by triangulation before it strikes. Binocular triangulation only works at the close ranges of about 25 mm at which the insect can strike with its raptorial forelegs. At farther prey distances, binocular vision is no longer effective because of the small distance between the eyes. At these greater distances, the mantis uses **saccadic tracking** to maintain the image of the prey on the **foveal region** of the compound eyes, a group of ommatidia in the center of each eye that is capable of high spatial resolution. The greater resolution in the foveas results from the larger facets and smaller angles between the neighboring visual axes. The saccades consist of rapid head movements that center the image of the prey on the foveas, followed by periods of no movement. By comparing coordinates of visual information from both the foveas as well as the ommatidia that surround the foveas of each eye, the mantis can still estimate prey distance by binocular triangulation at greater distances. Adult blowflies also use saccadic head movements during flight and while walking to reconstruct the three-dimensional landscape that lies before them. The resolution of the visual image can be degraded by rapid eye movements, so animals prevent blur by staring to fix their visual field. By minimizing saccades and remaining stationary in flight, hover flies maximize the detection of other objects in motion. Motion parallax and visual acuity are difficult to achieve at the same time.

Visual Defense

Insects use visual communication in a passive defense. Many lepidopterans bear conspicuous eyespots on their wings, which are the circular patterns that resemble the eyes of vertebrates. The lepidopteran, *Nymphalis io*, responds to the presence of birds by lowering its wings to expose its eyespots, and the sight of the eyespots releases escape behavior in the birds, often preventing the butterflies from being eaten. Butterflies whose eyespots have been removed experimentally are much more susceptible to bird predation than those that retain their eyespots. The noctuid moth, *Catocala*, has cryptic fore wings and brightly colored hind wings that are usually concealed. When grasped by birds, the moth exposes its hind wings and startles the bird sufficiently that it momentarily releases its beak and allows the moth to escape.

Bioluminescence

A prime reason for communicating visually is to be seen by a member of the opposite sex of the same species. An insect must be relatively conspicuous against the complex environmental background if it is to be visually identified by another individual. However, being conspicuous during the day also has its drawbacks, because a conspicuous visual signal is more apparent to predators. Some insects have consequently evolved the ability to communicate visually at night when predators are less active by producing the light themselves.

Although the function of light production may often appear to be obvious, its role is not always clear. In some cases, the emission of light may be incidental to the underlying biochemical pathways. As we will see, the oxygenation of the substrate luciferin results in a high turnover of molecular oxygen, and the reactions may have initially evolved as an antioxidant function. This oxygen detoxification was initially proposed as a mechanism for the evolution of bioluminescence in bacteria and has been subsequently proposed to explain how luminescence might have evolved in beetles. However, there are numerous antioxidant pathways that do not involve luminescence, and bioluminescence itself cannot be a prerequisite for oxygen detoxification. It is also possible that a new luminescent phenotype was responsible for the evolution of novel oxygenase functions. The function of luminescence in collembolans, which produce a light from their whole bodies, has never been determined but may be for defense rather than for mating. Larval bioluminescence may function for luring prey or aposematic signaling.

The capacity to produce light has evolved in at least nine insect families within five orders — the Collembola, Homoptera, Diptera, Dictyoptera, and Coleoptera — but bioluminescence occurs in more beetles, largely in the families Elateridae and Lampyridae, than in any other group. In the firefly beetles (Lampyridae), a yellow-green light with a wavelength between 550 and 580 nm is produced from a light organ derived from the fat body at the ventral tip of the abdomen, and the insects use this visual signal to find a mate. They may also use the light to illuminate landing sites.

Fireflies use two strategies to bring the sexes together. In some species, it is the female that produces specific flashes to attract males of the same species. In other species, the males initiate the flash signals, and when a receptive female is nearby, she returns the signal. Each species has its own flash pattern that serves as an isolating mechanism when species distributions overlap.

Flashing begins at twilight once the insects perceive a decrease in the intensity of ambient light to below a certain threshold. The fireflies are able to receive the flash signals with their superposition eyes that function well in dim light after they have been dark adapted. Both the flash duration as well as its spectral qualities are important to a receiver; the green and yellow sensitivities of the eyes are matched to the emission spectra of the light produced. As an example,

the males of the firefly, *Photinus pyralis*, begin patrolling open fields beginning at dusk, flying slowly and producing a 500-ms flash every 6s. The male then pauses for 2s, and if a female responds with a flash during this period, the male flies toward her and flashes again. The male ultimately reaches the female after a series of these flash exchanges.

Fireflies in the genus *Photuris* use a rather dishonest approach that employs visual communication for another purpose. In a strategy known as **aggressive mimicry**, they are able to mimic the flash patterns of up to five other species, attracting the naïve males that respond to the false signals, and instead of mating with them, they consume the males that respond. After their adult emergence, the *Photuris* females attract males of their own species, but once they mate, their behavior changes and only then do they begin to attract the males of different species in order to prey on them. Not all firefly species are luminous; the ancestral condition is to use pheromones to find mates, with luminosity as the more recent development.

The light organ, or lantern, of adult fireflies, is derived from modified fat body cells. Specialized light-emitting **photocytes** lie just beneath the transparent epidermis and cuticle and contain large numbers of photocyte granules in their cytoplasm along with numerous mitochondria localized near the tracheoles. The photocytes are arranged in rosettes that are separated by central cylinders containing the nerves and tracheal branches that run vertically through the photocyte layers (Figure 12.2). The tracheal system ends in a tracheal cell that in turn envelops a tracheolar cell and the nerve ending that innervates it. The nerves innervate only the tracheal end cell and not the photocytes. Underlying the photocytes are **dorsal layer cells** that contain white urate crystals and provide a reflecting surface for the light that is emitted.

Although flashing is initiated by a nerve impulse, the supply of oxygen to the photocytes controls the flash. During the dark phase, the tracheoles are filled with fluid, but nervous stimulation of the tracheoles stimulates their rapid fluid uptake and allows the oxygenation of the photocytes to occur (Figure 12.3). Nitric oxide (NO) gas is produced in the cells between the synapses and the tracheolar cells to briefly inhibit mitochondrial respiration, increase the O_2 levels, and allow oxygen to flow into the photocyte.

Light production results from the activity of the enzyme **luciferase**, when it oxidizes the substrate **luciferin** to produce the unstable compound **oxyluciferin** in a sufficiently excited state to yield a photon of visible light. Luciferin is a benzothiazoyl-thiazole derivative that is identical in all insect species that produce light. Luciferase has many forms, ranging from 545 to 550 residues and with an average molecular weight of 60kDa. Altering the amino acid composition of luciferase by substitution results in a shift in emission peaks of the light produced, and with these slight variations in luciferase structure, different species can produce a variety of wavelengths. Colors produced include yellow-green, blue, and red.

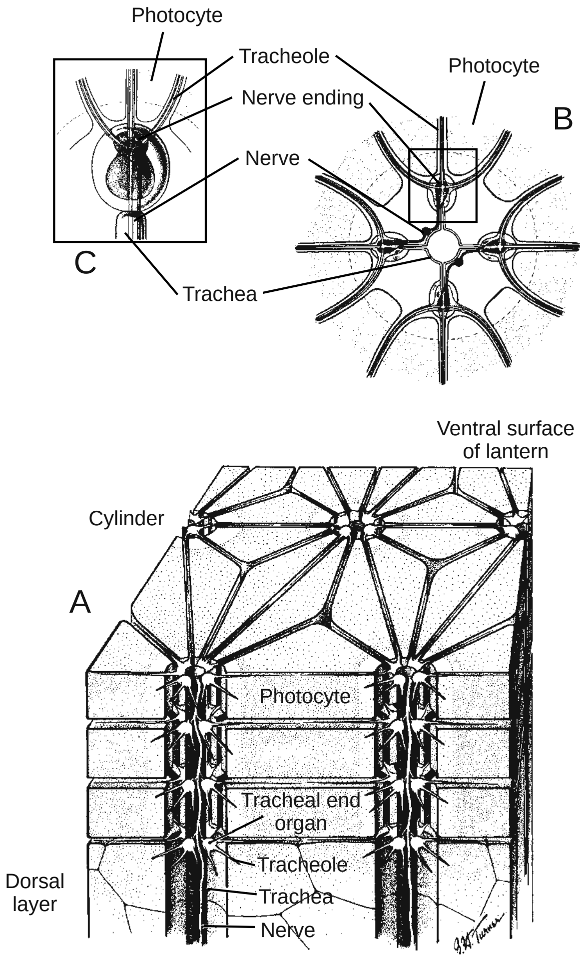

FIGURE 12.2. A. A cross section through the integument of the lantern of adult fireflies showing the cuticle at the top and the photocytes below. B. The arrangement of photocytes in a rosette around the central cylinder. C. A magnified view of the interface between the tracheole and nerve at the periphery of a rosette. From Buck (1966). Reprinted with permission.

Luciferase catalyzes the condensation of luciferin with ATP in the presence of magnesium, and the resulting adenylate, still bound to the enzyme, reacts with oxygen to form a dioxetane, a four-member peroxide ring (Figure 12.4). The ring decomposes across the peroxide bond to yield oxyluciferin in an excited state for the production of a photon of light. The light produced is cold and the luminescent reaction is very efficient, with a yield of over 90% relative to the consumption of the luciferin substrate.

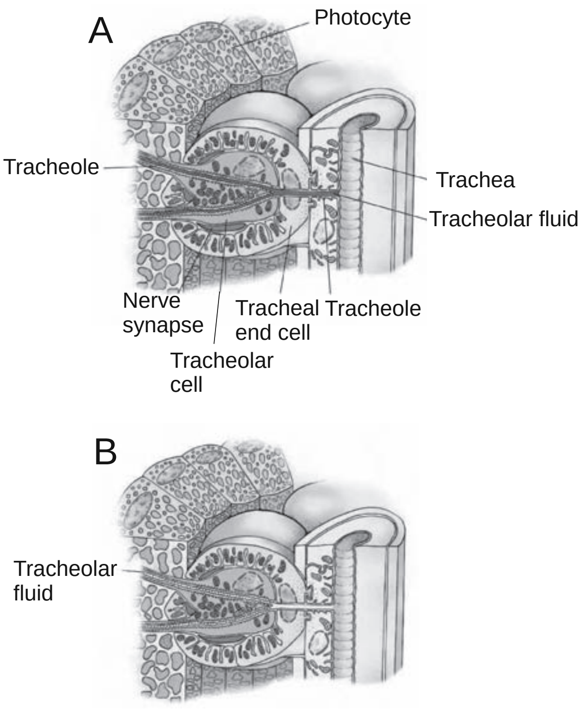

FIGURE 12.3. Changes in the length of tracheolar fluid controls access to oxygen by the photocytes. A. Shows the increased fluid length during periods of no light emission. B. The fluid length is decreased during the emission of light. From Timmins et al. (2001). Reprinted with permission.

ACOUSTICAL COMMUNICATION

Insects produce sound and vibrational signals through the air, water, or solid substrates, and they receive and interpret these signals by what we may infer to be hearing. Demonstrations of insect hearing mostly come from the orders Orthoptera, Diptera, Coleoptera, Lepidoptera, and Homoptera, but the production of sounds can easily be missed if only the human ear, with its relatively poor performance, is used as an analyzing instrument. Thus, there are undoubtedly many more instances of acoustical communication in insects that we may be unaware of. Vibrations may be produced by a variety of mechanisms and through a variety of substrates to ultimately reach another insect and change its behavior.

Sound Production by Percussion

Sounds can be produced by **percussion**, involving the bringing together of two rigid structures. The male Australian whistling moth, *Hecatesia thyridion*, has the

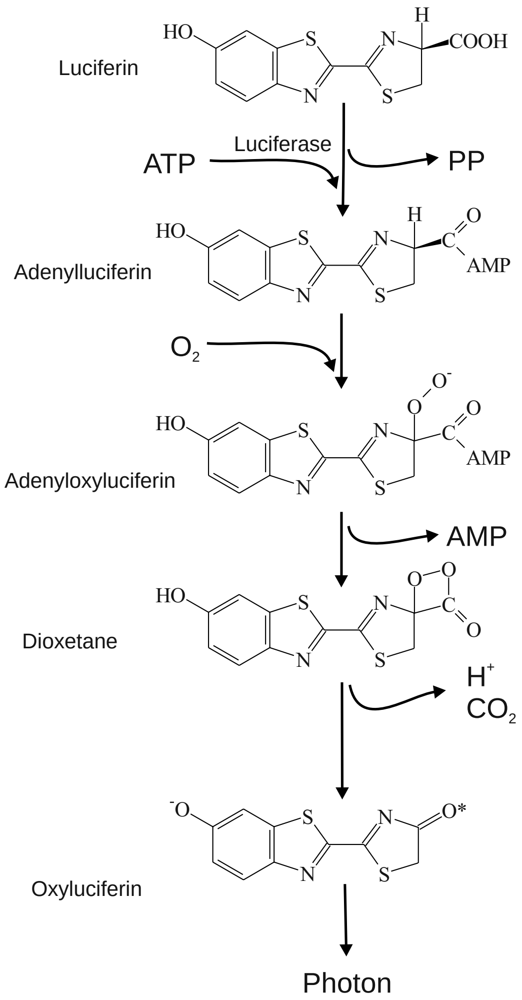

FIGURE 12.4. The oxidation of luciferin to release a photon of light. From Wood (1995). Reprinted with permission.

costa of its fore wings modified into small knobs called **castanets**. As the wings come together at the top of the wing stroke, these castanets strike each other and produce sounds that resonate through the wings and attract females.

The production of sound can also occur by the striking of a body part against the substrate. Pscopterans and plecopterans can tap their abdomens against the ground to make drumming sounds; in plecopterans, a peg or hammer is located on the seventh to ninth abdominal segments that strikes the substrate. Using their heads, the social ants and termites can drum on the ground to warn colony

members of danger. Males of the katydid, *Meconema thalassinum*, produce a drumming sound by tapping one of their hind tarsi on the ground.

Sound Production by Vibration

Sounds can be produced by using muscles to directly vibrate a membrane. The special membrane is called a **tymbal** and functions much like the lid of a tin can when it is pressed and released with a finger. The tymbal mechanism is present in many homopterans and is most utilized in male cicadas where they appear as paired structures that are borne on the first abdominal segment. Some arctiid and noctuid moths bear a tymbal on either side of the metathorax to produce sounds as a defense against echolocating bats. Cicadas are believed to be the loudest insects on the planet, with special muscles that allow the rapid contractions to occur. These synchronous superfast muscles operate at frequencies that are two to three times that of conventional synchronous skeletal muscles and have a special organization that allows fast calcium triggering and makes sufficient ATP available.

The chitinous membrane comprising the tymbal is normally bowed outward and is surrounded by a sclerotized ring. The membrane may contain ribs of varying length that when buckled inward produce a loud click. The tymbal muscle is attached to the center of the tymbal, and when the muscle contracts, it pulls the membrane rapidly inward and then relaxes to release it, in the process moving the air and producing a sound as it moves in either direction (Figure 12.5). Tracheal air sacs may be present behind the tymbal to produce a sympathetic resonance and prevent other internal tissues from damping the sounds. The volume of the tracheal sac of the Australian cicada comprises 70% of the insect's abdominal volume. In those species that bear tymbals lacking these sacs,

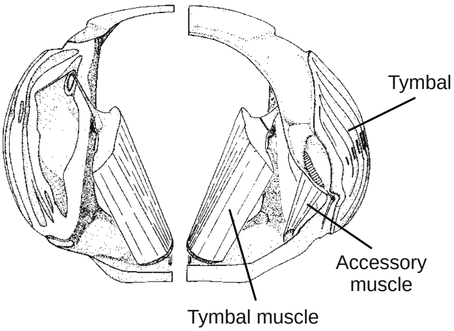

FIGURE 12.5. The tymbal on the outside body wall and its musculature. From Simmons and Young (1978). Reprinted with permission.

much softer sounds are issued. The males of some cicada species produce double pulses from the inward movement of the tympanum that is caused by the buckling of the tymbal in two stages. The two tymbal muscles on either side contract alternately at a frequency of 120 hertz, producing a final signal of about 240 hertz. Accessory muscles that attach to the rim of the tymbal and increase its convex shape are able to modulate the volume of the sounds that are produced. The energy required to move the tymbal and the intensity of the sound produced is increased by altering the curvature of the tymbal when the muscle contracts. The male cicada cry has been recorded at 100 decibels at one meter away, with only about 30 decibels required to stimulate a female.

Female cicadas bear a tympanum on the ventral portion of their second abdominal segment that functions as an ear. It consists of a thin cuticular membrane stretched over a tracheal air chamber, with a chordotonal organ attached. The sensory apparatus that connects to the tympanum is composed of an unusually large number of receptor cells, up to 2100 in each ear, allowing the female to distinguish the frequency components of male songs.

Insectivorous bats use ultrasonic pulses to find their food at night by echolocation. Bats can first detect a moth about 6 m away, but with its tympanum, the moth can register the bat's approach at the greater distance of about 35 m and execute evasive maneuvers to escape predation. Praying mantids also perform evasive behaviors depending on the distance of the bat. In addition to evasive behavior, some arctiid and ctenuchid moths produce their own ultrasonic clicks from their metathoracic tymbals that may either signal the moth's distastefulness to the bat or jam the bat's ultrasonic detection system and interfere with its echolocation.

Many insects produce vibratory sounds by **stridulation**, the rubbing of two body parts against each other. One body part that contains a series of teeth or corrugations is referred to as the **file** or **pars stridens**, and the opposing part may consist of a peg or tooth that is known as the **scraper** or **plectrum** and is dragged across the file to create the sounds. Crickets have a row of chitinous teeth on the ventral side of a wing vein that acts as the file, with the scraper formed from the inner edge of the elytron (Figure 12.6). Sounds are produced during the closing of the wings when the scraper on one side of the wings is moved against the file on the other side of the wings. A wing area known as the **harp** is corrugated and is driven to vibration by the stridulation, radiating the sounds that are generated. A **mirror** may also be present, which is an area consisting of a thin cuticle that is supported by surrounding wing veins and vibrates when it is distorted by the scraper-file interaction.

Male mole crickets modify their burrows into acoustical chambers, creating a horn that increases the efficiency of their harps' vibrations to attract females (Figure 12.7). A song produced when the cricket is outside its burrow is significantly less intense than one produced while in the burrow. The song produced outside is only about 4% of the intensity of one produced within the burrow,

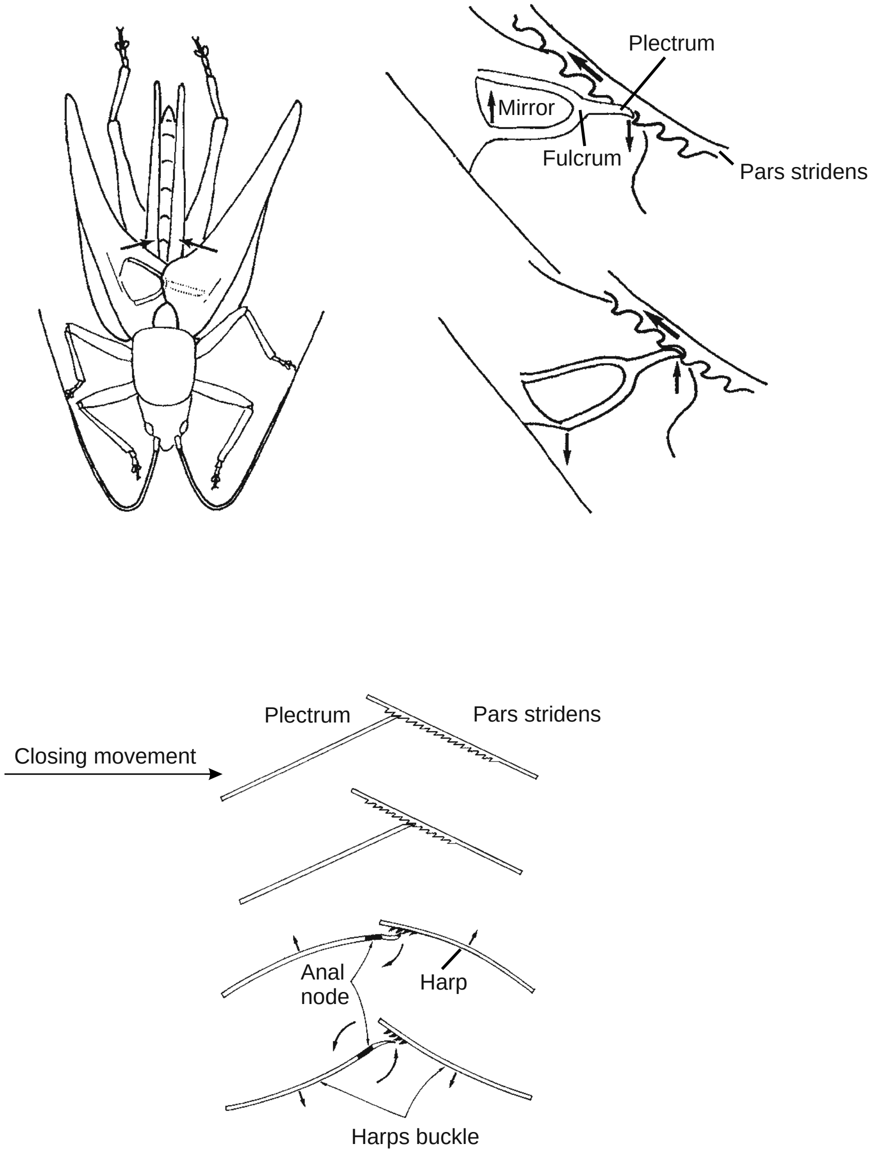

FIGURE 12.6. (*Top*) The stridulatory apparatus of a katydid. The mirror frame vibrates as the file on one elytron is moved across the plectrum. (*Bottom*) The stridulatory apparatus of a cricket. From Ewing (1989) and Bennet-Clark (1989). Reprinted with permission.

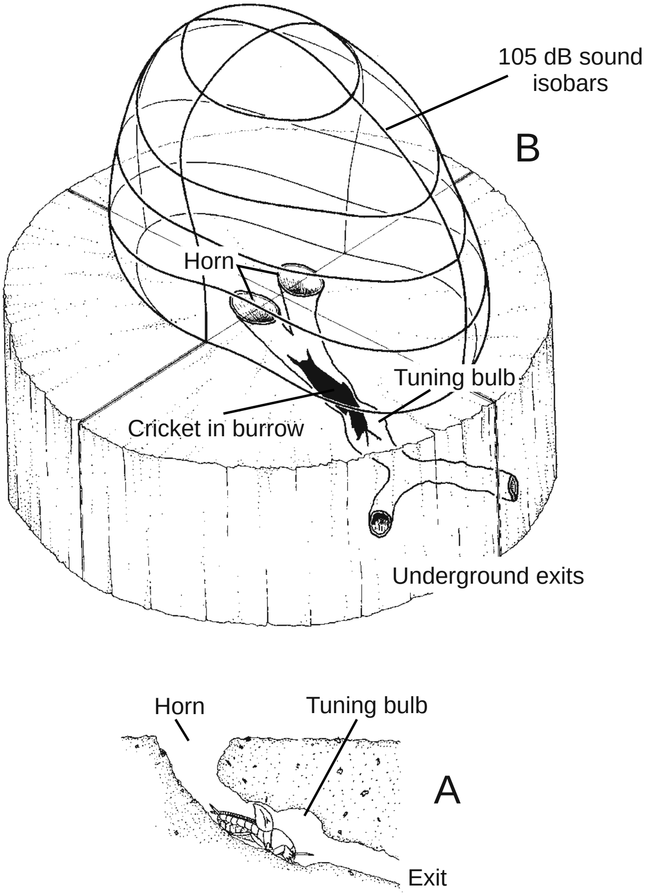

FIGURE 12.7. A. A cross section of a mole cricket burrow. B. A three-dimensional view of the acoustical chamber that amplifies the stridulations. From Bennet-Clark (1989). Reprinted with permission.

which is amplified by the chamber and can be heard by humans up to 600 m away.

Beetles have a variety of mechanisms with which to stridulate. Some have a file at the top of their heads that is scraped by a ridge under the anterior of the pronotum. In others, the file is located under the head and the scraper located on the anterior margin of the prosternum. The file and scraper may both be located on the thorax or abdomen, or the file alone on the thorax or abdomen and the scraper on an appendage (Figure 12.8). Leg stridulation occurs in some grasshopper families. The hind femora bear stridulatory pegs that are rubbed against a sharpened wing vein (Figure 12.9).

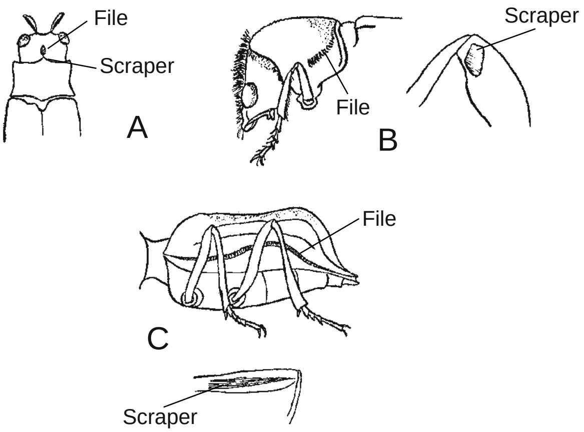

FIGURE 12.8. Various stridulatory structures on coleopterans. (A). A file at the top of the heads that is scraped by a ridge under the anterior of the pronotum. (B). A file located under the head with the scraper on the anterior margin of the prosternum. (C). The file located on the abdomen and the scraper on the leg.

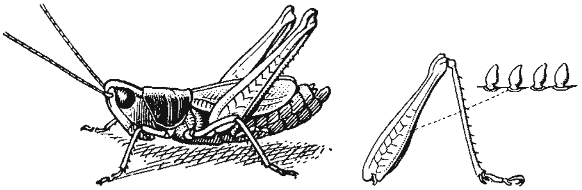

FIGURE 12.9. (*Right*) The stridulatory pegs on the legs of a grasshopper. From Haskell (1961). Reprinted with permission.

Some tachinid flies that are parasitoids of male crickets use the songs of their cricket hosts to locate them. Rather than possessing a typical Johnston's organ that is used to detect vibration in many other dipterans, these flies have evolved a tympanum much like those of the cricket. With this tympanum tuned to the songs of its host, the female fly is able to locate the male cricket as it calls and deposit its eggs on or nearby him. In some cricket populations, an adaptive response to this challenge was a shift to silent communication with females. Although not an example of a signal originating with an insect, the calling song of *Hyla* tree frogs attracts mosquitoes in the genus *Corethrella* for a blood meal.

South American katydids in the genus *Arachnoscelis* stridulate to produce a calling song of 129 kHz, the highest note known to be produced by an arthropod. The file is located above a wing vein and the scraper is situated on the hind margin of the fore wing. When the fore wings are scraped together in a low velocity movement, the elastic energy stored in the cuticle rapidly deforms and unbends the scraper to yield a significantly higher frequency pulse.

The displacement of air during the rapid movement of the wings during flight creates sounds, and some insects have used this mechanism as a means of communication. In those mosquito species that swarm, the males hover over a conspicuous marker at sunset, and when females enter the swarm, the males are attracted to them by the tone produced by the female's wing-beat frequency. *Drosophila* males and females produce pulses and bursts of sound from their incomplete wing-beats while they walk. The male's song stimulates the female to engage in the courtship ritual. Honeybees also produce pulses by vibrating their wings during the waggle dance to inform other workers about the location of resources.

Sound Production by Substrate Vibration

Insects can use plants as transmission channels for vibrational communication. Insect herbivores commonly use plant stems, leaves, and roots to communicate signals over relatively short distances of less than 2 meters. It is commonly used for courtship communication and locating other individuals of the same species, but it can also be used as warning signals for nearby predators and to recruit other individuals to new feeding sites. The low-frequency signals, usually indicative of a much larger animal, cannot be sent through the air but can be efficiently sent through a solid substrate. The senders may drum the plant with their tarsi, or as for the African tok-tok beetle, *Psammodes striatus*, tap the substrate with its abdomen. Blind worker termites can assess the quality of the wood they eat by the vibrational signals they receive from other termites, and as mentioned previously, soldier termites generate alarm signals by drumming their heads on the substrate. Acoustic signaling between caterpillar larvae occurs by mandibular drumming and scraping on leaf surfaces. The subgenual organs in the legs of receivers and their various antennal receptors are the most sensitive to substrate vibrations. Some bird predators use the acoustical cues from their insect prey as a means of locating them.

Sound Production by the Expulsion of Air

Cockroaches in the genus *Gromphadorhina* expel air through their tracheal systems in response to disturbances and during courtship. The fourth abdominal spiracle is modified with an inner constriction and a large opening to the outside, and as the air exits, the spiracle creates a hissing sound. All spiracles but the fourth are closed when the abdominal muscles contract so that air is vigorously forced out of the single open pair. The tsetse, *Glossina*, has been observed making

ultrasonic sounds during feeding and mating that have been attributed to the release of air through the tracheal system when the tracheae supplying air to flight muscles are contracted.

TACTILE COMMUNICATION

Tactile signals are used for short-range communication, generally for aggressive or sexual encounters. Aphids engage in violent kicking when they are crowded, physically displacing other aphids to gain more space for themselves. Many insects must touch their mates before copulation can begin, receiving close-range chemical signals that release the required stepwise behaviors. Contact chemoreception is especially important in dipteran mating systems, with female cuticular hydrocarbons that are perceived by touch serving as essential behavioral releasers. Males of both the house fly, *Musca domestica*, and the tsetse, *Glossina morisitans*, use visual stimuli as initial attractants, but as they approach the females, cuticular hydrocarbons act as attractants that are necessary for the close-range stimulation of the males. The male cuticles of both of these dipterans contain substances, the **abstinons**, that have anti-homosexual activity when contacted, deterring the males from attempts to copulate with other males. The crane fly, *Tipula oleracea*, requires a rigid sequence of tactile communications in order to mate, including stereotyped leg movements. Courtship can be broken off if any of the tactile stimuli are perceived at the wrong time or missing altogether.

The social ants and termites live in darkened underground galleries and must use cues other than visual ones to recognize nestmates. Individuals of both groups may produce colony-specific hydrocarbons that are detected by a sweep of the antennae or palps across the cuticular surface of another individual. Each colony has a chemical signature that is read by touch and that triggers aggressive behaviors if it fails to match the signature that is expected.

Schistocerca gregaria desert locusts undergo a phase change when the immatures are reared under crowded conditions. Although their solitary phase is characterized outwardly by cryptic coloration and a reluctance to interact with others, their phase polyphenism includes a suite of characters that transforms them into conspicuously colored gregarious individuals whose reproductive development and fecundity, endocrine physiology, and behavior are markedly different. Not all the characteristics of this polyphenism change at the same time but instead develop gradually. The most important stimulus for the density-dependent change toward gregarious behavior is the stimulation of tactile sensory receptors on the hind femur when individuals become concentrated as population numbers increase. Stimulation of these tactile hairs activates a neuronal circuit involving nerve 5B and the locust's central nervous system (Figure 12.10).

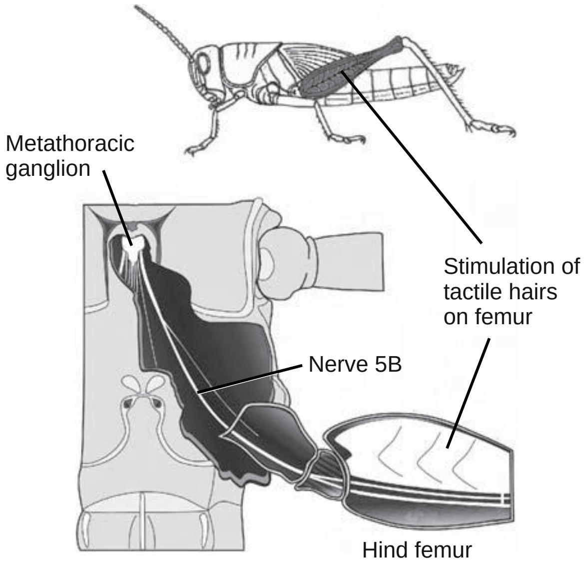

FIGURE 12.10. Stimulation of the tactile hairs on the femur of locusts activates neural circuitry in the metathoracic ganglion via nerve 5B. From Rogers et al. (2003).

CHEMICAL COMMUNICATION

Insects make an extensive use of chemicals that are released into the surrounding medium for communication. They employ chemicals for communicating to find a mate, gather together, provide others of their own species with the location of food, identify nestmates, and defend themselves against predators. Insects live in a complex chemical world, with chemical cues governing much of their behavior and most of their interactions. The classification of the chemicals that insects use to communicate is based on the functional roles of those chemicals in the interactions they mediate.

The chemicals that mediate physiological or behavioral processes can be categorized as either **hormones** or **semiochemicals**. Hormones, as we have seen, are produced by one organism and mediate physiological reactions within that producing organism. In contrast, any chemical that mediates an interaction between two organisms, whether of the same or different species, is a semiochemical (Greek: *semeon*, a signal). Semiochemicals are further divided into two categories based on whether the use of the chemical is between members of the same or different species. **Pheromones** are semiochemicals that mediate intraspecific interactions, and **allelochemicals** are those that mediate interspecific interactions (Figure 12.11).

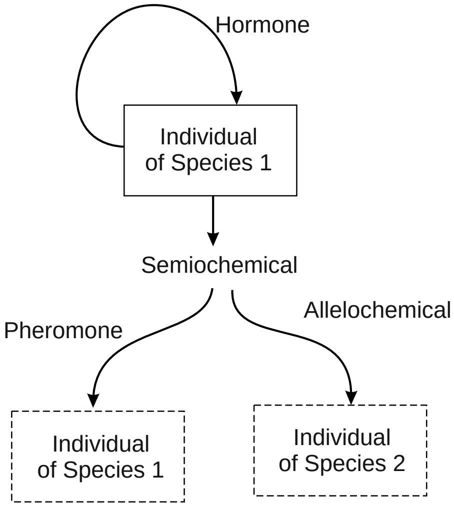

FIGURE 12.11. The actions of hormones and semiochemicals. Hormones are produced by an individual and act on cells within its body. Semiochemicals are chemicals that mediate the interactions between two organisms. If the organisms are of the same species, the chemical is called a pheromone. If the organisms are a different species, the chemical is termed an allelochemical.

PHEROMONES

Chemical communication between single-celled protozoa was certainly well established before multicellular organisms evolved. The hormones that organize and coordinate the development of multicellular animals may thus be the intracellular equivalent of the pheromones that the unicellular organisms employ, suggesting that pheromones may be the chemical ancestors of hormones. Pheromones are produced from **exocrine glands**, which are modified epidermal cells, and are transmitted to another individual of the same species.

The first insect pheromone, bombykol, was isolated in the mid-1950s from more than 300,000 *Bombyx mori* silkworm moths. From this large amount of biomass, only 5.3 mg of the active product was obtained. To identify the pheromone components active in the boll weevil, *Anthonomus grandis*, 4 million insects were processed along with 55 kg of their fecal material. With improvements in analytical instrumentation, a large number of insects was no longer necessary, and the isolation and identification of insect pheromones has since proceeded at an ever-increasing rate. The pheromones for more than 3000 insect species have been identified, with more than 1700 from lepidopterans alone. A number of **parapheromones**, which are analogs and mimics of the natural products, have been synthesized for use as tools in pest management.

Pheromones were first called ectohormones because they were secreted by glands and had the physiological effects of a hormone, but unlike hormones they

were produced outside of the organism that they affected. However, hormones are substances secreted internally, so the name "ectohormone" was thus a contradiction in terms and the name "pheromone" (Greek: *phereum*, to carry; *horman*, to excite) was adopted instead. **Pheromones** are chemicals produced by specialized glands that are secreted to the outside by one animal and have a specific effect on another individual of the same species. They are typically active in extremely small concentrations and usually as a mixture of compounds in a species-specific pheromone blend. The individual components in the blends are often common to several species, with the precise proportions of the individual components conferring the species specificity. It is not uncommon for geographic variants of a species to produce significantly different proportions of the pheromone components. There is one compound, (*Z*)-7-dodecen-1-yl acetate, that is used by more than 126 insect species as a component in their pheromone blends, and incredibly it is also a component of the pheromone that female Asian elephants produce in their urine to broadcast their readiness to mate. On another continent, ovulatory female African elephants produce several aggregation pheromones of bark beetles, including frontalin and *exo*- and *endo*-brevicomin. How the elephants use these signal molecules has yet to be identified.

For a chemical signal to be distinct in the chemically noisy environment, one strategy is to use one complex component that would never appear otherwise, and indeed, several species appear to employ only a single component in their pheromones. The American cockroach, *Periplaneta americana*, uses periplanone B alone to attract males from a distance. It is a bit unfortunate that bombykol, the first pheromone to be identified, originally appeared to consist of a single component. Using this as a model for much of the future research on insect pheromones, many subsequent investigations looked for single-component pheromones in other species. However, many of these single components failed to show the same activity as an intact insect did in bioassays, and as the pheromones from more insects were identified, it became apparent that many of the same chemical components were present in different species. When electroantennograms showed responses of receptors to the single components, but the whole insects perceiving them failed to display the behavior, it was eventually realized that most pheromones consisted of blends of different compounds, and the species specificity that is present in these pheromones results from the specific pattern of the blended components that the receiver collects. Even what was initially believed to be a single component in bombykol ultimately turned out to be a multiple component system. Pheromones produced by two species of the leaf-rolling moth, *Archips argyrospilus* and *A. mortuanus*, are good examples of these blends. Both species produce a pheromone with the same four components, but with respective proportional blends of 60:40:4:200 and 90:10:1:20. The ratio of the first two components is the most important for maintaining the reproductive isolation between these species. The specificity of most pheromones lies in their proportional blends.

The variety of pheromone blends evolved as a result of major shifts in the production of the constituent components rather than gradual changes in the proportions of the chemicals involved. The high species specificity of pheromone blends suggests there is strong selection against their modifications and argues against the evolution of blends through small changes. Large shifts in the blends of many species can be accomplished by the activity of a single gene, making it relatively easy to abruptly shift the chemical code. Because pheromone production often occurs as a result of a few recurring steps using the same enzyme, a change in the enzyme can result in major changes in the pheromone blends. This, of course, also must be accompanied by a change in the receptors or a relative nonspecificity in the receiving sex. This has implications for the chemical control of economic pest species using pheromone mimics to trap them, because pheromone resistance can be theoretically acquired easily.

As with hormones, pheromones can be broadly subdivided according to their mode of influence. **Releaser pheromones** stimulate an immediate and reversible behavioral response that is mediated by the nervous system soon after the receiver perceives it. Several insect behaviors are released by pheromones. In contrast, **primer pheromones** mediate a fundamental physiological change in the receiver that reprograms it for an altered response, acting directly on the nervous system or some other physiological system. The response may not be immediate as for a releaser, and it may be a novel response that was not expressed previously for that same stimulus (Figure 12.12). Primers are utilized mostly by social insects to regulate a variety of social interactions. Releaser components may also serve as primers. In the honeybee queen, 9-oxo-(*E*)-2-decenoic acid is both a component of her pheromone blend that establishes the retinue response in workers and the primer pheromone that suppresses their ovarian development.

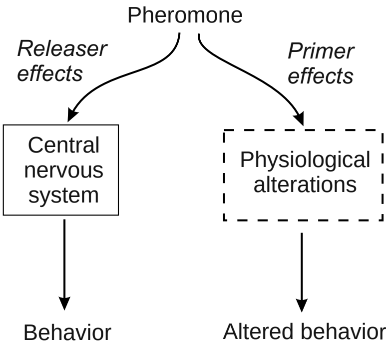

FIGURE 12.12. The releaser and primer effects of pheromones. A releaser pheromone acts on the central nervous system and causes the immediate release of a behavior. A primer pheromone alters the physiological state of the receiver, causing another behavior to be expressed.

RELEASER PHEROMONES

Releaser pheromones make up a diverse group of chemicals and are best subdivided on the basis of the functions they serve. The most common functional categories include **sex pheromones**, **aggregation pheromones**, **alarm pheromones**, **trail pheromones**, and **dispersal** or **spacing pheromones**.

Sex Pheromones

Sex pheromones are chemicals produced by insects of one sex that elicit a behavioral response in members of the opposite sex. They advertise the presence of an individual and lure others of the species for mating. The mechanisms by which the pheromones are produced and received vary considerably among insects. If the perception of the pheromone releases a long-range searching behavior, it is called a **sex attractant**. If it facilitates closer-range courtship behavior or copulation, it may be referred to as an **aphrodisiac**. Either the male or the female of the species may produce the pheromone, and in some cases, mating behaviors are released by pheromones that are produced by both of the sexes. Volatile sex pheromones are usually released at certain times during the insect's life cycle and certain periods of the day rather than continuously during an individual's life, and their release may be terminated once the individual mates. **Calling behavior**, which consists of a particular posture and the eversion of pheromone glands to allow the pheromone to evaporate, occurs in many sexually mature insects.

The use of sex pheromones by moths has probably been studied the most intensively of any insect. Typically, the female releases a long-distance attractant that arouses the male, which becomes airborne and flies upwind into the chemical plume. The plume is not a continuous swirl of increasing concentration of pheromone molecules but rather is more like the smoke from a chimney in a gentle breeze. Filaments of the plume drift downward, interspersed with air that is free of any pheromone molecules, so the male encounters a discontinuous stimulus trail. The pulsed signals are necessary to find the female; with a continuous strong signal, the pheromone receptors become adapted and the male terminates his flight. He maintains a zigzag flight pattern in and out of the plume as he enters and leaves the trail until reaching the female. After landing, the male may release a short-distance aphrodisiac pheromone to attract the female, and copulation follows.

Aggregation Pheromones

Unlike sex pheromones that act on only one sex, **aggregation pheromones** induce group formation by bringing many individuals of both sexes together.

Their activity may resemble that of sex pheromones because they often increase the probability that copulation will occur in the population. They are produced mainly by coleopterans as a defense against predators and to overwhelm the resistance of a host tree. For example, female bark beetles are the first to bore into a tree and release an aggregation pheromone that along with the host terpene attracts both males and females. The sexes are brought together for mating, but the large number of individuals present also benefits the beetles by overcoming the tree's resin and toxin defense mechanisms. The tree is also inoculated with fungi that the beetles carry in specialized structures. Coccinelid beetles produce an aggregation pheromone that attracts large numbers of males and females to overwintering sites, and their aposematic coloration is enhanced and predators discouraged when the brightly colored insects are aggregated. The honeybee queen produces an aggregation pheromone from her mandibular glands that is responsible for the retinue of workers that attend to her, and it also stabilizes the colony around the queen when it swarms.

Alarm Pheromones

Alarm pheromones are produced mostly by social insects to warn other colony members of danger and to recruit for colony defense. It is certainly more adaptive and effective for a species to mount a collective response to some traumatic stimulus rather than to mount an individual response. In the honeybee, the release of isopentyl acetate and more than 20 other substances by alarmed workers in the act of stinging releases a frenzied attack by other workers. Africanized honeybees produce 3-methyl-2-buten-1-yl acetate in their sting gland. Ants synthesize their alarm pheromones in their mandibular glands and release them when attacking prey to recruit other colony members. Weaver ant workers additionally strike the leaf when disturbed to attract other workers to the disturbance. Aphids and treehoppers secrete alarm pheromones that cause them to fall off the plant and escape possible predation. The green peach aphid, *Myzus persicae*, secretes the alarm pheromone (E)-β-farnesene from its cornicles when attacked by predators. Alarm pheromones generally consist of low-molecular-weight, highly volatile compounds that easily spread throughout a colony yet evaporate quickly to terminate the aggression when the danger no longer exists.

Trail Pheromones

Trail pheromones are also found mostly in social insects, including ants, termites, bees, and wasps. When a worker locates a resource, she lays down a trail when returning to the colony that other workers can use to find the resource. Flying insects use trail pheromones to stimulate colony members to

enter the hive. Bees mark the nest entrance with products from the Nasonov gland that induce workers to enter. There is also evidence for a pheromone deposited from the tarsi of bees and wasps that may serve as a trail pheromone at the nest entrance.

Venomous ants use their sting to lay down a product of the poison glands as a trail pheromone, while nonvenomous ants synthesize their pheromone in Dufour's gland or in the gut. These terrestrial trail pheromones serve as a sensitive index to the amount of food present at a distant location, because each worker returning from the resource adds to the trail's intensity. Once the food is exhausted, returning workers no longer lay down a trail, and it soon dissipates. Trail pheromones tend to be more stable than alarm pheromones, but they are still relatively volatile, a necessity if trails that are no longer informative are to be avoided.

Trail pheromones appear to have arisen as metabolic by-products that were eventually adapted as signals and may be exceptions to the rule that pheromones exist as specific component blends. A single component, 3-ethyl-2,5-dimethylpyrazine, is present in the poison glands of several species of *Myrmica* and is able to induce trail-following in all those species, but there are some other species that do indeed use multiple components.

Epideictic Pheromones

Spacing pheromones are also known as **epideictic pheromones**. They maintain the densities of individuals attempting to exploit an exhaustible resource to numbers that are below their carrying capacity. Female tephritid fruit flies oviposit on the flesh of fruits where the larvae develop and mature. Some of the fruits are only large enough to support a single larva. Immediately after she oviposits, the female circles the fruit as she trails her ovipositor on its surface and deposits a pheromone that deters other females from laying eggs on that fruit. Female bark beetles are the first to attack a tree and produce an aggregation pheromone that attracts both males and females. Along with the increase in the number of aggregating males comes an increase in the epideictic pheromone they produce, and this prevents subsequent males and females from landing on that resource so that it is not overpopulated.

Funeral Pheromones

So-called **funeral pheromones** are produced in dead ants that stimulate other live colony members to remove them to a pile outside the nest. When an object is covered with an extract of the saturated fatty acids that are thought to be responsible, it is treated as if it were a dead ant.

PHEROMONE SYNTHESIS AND RELEASE

The synthesis of pheromones may occur throughout the adult insect's life, but release of the synthesized pheromones generally occurs only during certain environmental and physiological circumstances. Bark beetles only release their pheromones during the day, whereas nocturnal moths engage in calling behavior and pheromone release only at night. The larvae of the moth, *Antheraea polyphemus*, feed only on oak leaves, and adults require the presence of *trans*-2-hexanol from the oak leaves in order to call. Females that mate more than once release pheromones periodically, but females that mate only once usually terminate their pheromone release after mating.

Pheromones are generally produced by modified epidermal cells that can be found in various places throughout the body. These are often clustered into groups designated as **exocrine glands**, secretory glands that direct their products to the outside of the organism. Exocrine glands are generally classified into two major types depending on their structural organization. Type I glands appear to be most immediately derived from epidermal cells, formed by a simple epithelial layer or lining an internal reservoir that temporarily stores the secretions. Type II glands consist of both secretory and duct cells, with the secretory components far removed from the cuticular epidermal cells.

The pheromone produced by adult male *Schistocerca gregaria* locusts that affects the rate of maturation of other nearby males and females originates in secretory cells dispersed throughout the epidermis. Many female lepidopterans release pheromones from type I glands that are modified epidermal cells located within the intersegmental membrane between the eighth and ninth abdominal segments. These glands may be deeply invaginated, but when the female displays calling behavior, the glands are everted from the body cavity and the volatile secretions are allowed to evaporate to the outside. Eversible brush structures on the sternites at the tip of the abdomen of male noctuid moths spread out fanlike when the male engages in courtship. These so-called **hair pencils** consist of hypertrophied trichogen cells accompanied by long hairs. Some glands cells are internal, such as Dufour's gland in ants and the frontal gland of termites that produce alarm pheromones. The gland cells produce pheromones that are stored in a reservoir that is lined with cuticle. The type II mandibular glands of bees produce alarm pheromones, sex pheromones, and the queen substance that inhibits ovarian development in the workers of a colony. In some stinging ants, the poison gland of the sting apparatus produces a trail pheromone.

Insect pheromones consist of an extensive variety of chemical components. In general, signals that require a rapid dispersal, such as the alarm pheromones, utilize smaller molecules, and signals requiring a more persistent exposure, such as sex pheromones, utilize larger molecules. Their activity is determined by the molecule's shape, size, functional groups that are present, and degree of unsaturation. Most work on mechanisms of pheromone synthesis has been focused on

lepidopteran sex pheromones. The components of the blends are produced from fatty acids, generally with 10, 12, 14, 16, or 18 carbons in a straight chain; alcohols, acetates, or aldehydes as single functional groups; and one, two, or three double bonds.

The sex pheromone of the cabbage looper, *Trichoplusia ni*, consists of a blend of saturated and unsaturated acetate esters of fatty acids. Beginning with fatty acids, the six components of the blend are produced by the action of a Δ-11 desaturase, an enzyme unique to lepidopteran sex pheromone glands, along with chain-shortening reactions, reduction, and acetylation. A complex blend of products is thus produced by employing only a few recurring biosynthetic steps, and pheromone evolution can occur abruptly with a sudden shift in synthesis. A large shift in pheromone blends occurs between the Asian corn borer (ACB) and the European corn borer (ECB), two related species in the genus *Ostrinia* with genomes that both potentially encode several desaturases for pheromone synthesis. Beginning with hexadecanoic acid, each species follows a different route to synthesizing its respective pheromone: Z/E12-14:OAc for the ACB and Z/E11-14:OAc for the ECB (Figure 12.13). In the ECB, the Δ11-desaturase transcript is the only functional product of its desaturase genes, whereas in the ACB, only the Δ14 desaturase gene is functional. A major shift in pheromone synthesis occurred as a result of the activation of previously nontranscribed genes in the pheromone glands.

The release of sex pheromones by most moths occurs during the evening hours when they are reproductively competent, and it is controlled by both an endogenous circadian rhythmicity and physiological factors such as mating status. In those species whose females engage in calling behavior, pheromone synthesis is regulated hormonally by a neuropeptide from the subesophageal ganglion, the

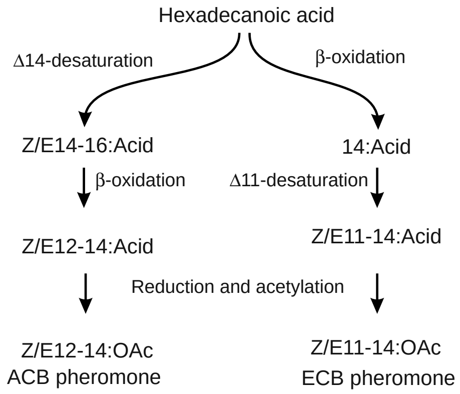

FIGURE 12.13. Pheromone blends in the Asian corn borer (ACB) and the European corn borer (ECB) result from the activation of different desaturase genes that are present but not transcribed in each of the species.

pheromone biosynthesis activating neuropeptide (**PBAN**), a 33 to 34 amino acid peptide that regulates pheromone production by activating enzymes involved in the synthesis of the final product. PBAN was first identified in female *Helicoverpa zea* moths in 1984 but has since been reported from at least six other species. Several other peptides with the common C-terminal sequence (FXPRL-NH_2) also stimulate sex pheromone synthesis, and all of these have been categorized as belonging to the PK/PBAN family of peptides. The members of this family play a number of other roles in insects, including melanin synthesis, gut contractions, and diapause. A single gene, DH-PBAN, is responsible for encoding a precursor polyprotein that is cleaved to result in five neuropeptides, two of which are PBAN and diapause hormone (DH). The functions of the other three peptides are not yet known.

In the silkworm, *Bombyx mori*, an embryonic diapause is induced by DH, and in several heliothid moths, DH terminates their pupal diapause. The diapause in these species is initiated in response to short day length during the larval period, caused by the failure of the brain to produce PTTH and the prothoracic glands to produce ecdysone. DH acts on the prothoracic gland to produce ecdysone, whose absence maintains the pupal diapause. The *Bombyx* embryonic diapause can also be induced by PBAN.

The mechanism of PBAN activation varies by species. In *Helicoverpa zea* and *Bombyx mori*, PBAN binds to a cell membrane receptor on pheromone gland cells that activates the influx of calcium. After first binding to calmodulin, the calcium stimulates a phosphatase that dephosphorylates and activates a reductase as the last step in bombykol pheromone biosynthesis in *Bombyx* (Figure 12.14). In *Helicoverpa*, the calcium-calmodulin activates adenylate cyclase resulting in cAMP production, which then activates a kinase to stimulate the enzyme acetyl-CoA carboxylase, the first in the synthetic pathway in that species (Figure 12.14). In the moth *Sesamia nonagrioides*, PBAN activates an acetyl transferase that produces the major component, Z11-hexadecenyl acetate (Figure 12.15).

The targets of PBAN activity are the glandular epithelial cells on the intersegmental membrane of the ovipositor tips, where pheromone production occurs in the females of several moth species. Some female moths terminate their production of sex pheromone once they mate because the release of PBAN from the subesophageal ganglion is suppressed after mating. The signal that mating has occurred is transmitted from a spermatheca full of sperm to the subesophageal ganglion by a neural signal in some species and by a humoral signal from the spermatophore in others. A pheromone suppression peptide produced by male *Helicoverpa*, *Hez*-PSP, suppresses pheromone production after mating.

Juvenile hormone has been implicated in the control of pheromone biosynthesis in coleopterans. The aggregation of bark beetles on coniferous host trees is released by the pheromones produced by the attacking beetles in their defecations. In the bark beetle, *Ips*, feeding on the *Pinus* tree host activates the corpora allata to produce JH III, and this activates two key enzymes — 3-hydroxy-3-

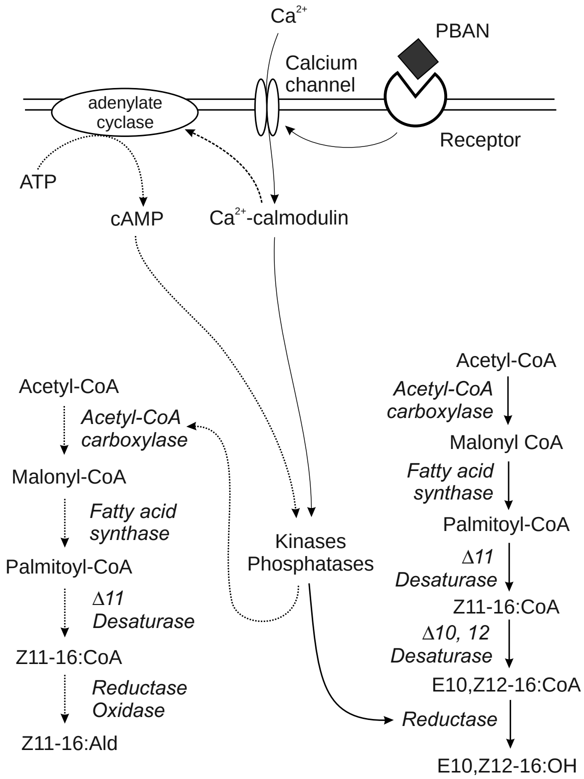

Figure 12.14. The activation of key enzymes in pheromone synthesis by pheromone biosynthesis activating neuropeptide (PBAN) in two lepidopteran species. (*Right*) The phosphatase dephosphorylates and activates a reductase as the last step in bombykol biosynthesis in *Bombyx*. (*Left*) cAMP activates a kinase that activates the enzyme acetyl-CoA carboxylase, the first in the synthetic pathway in *Helicoverpa*.

methylglutaryl-CoA (HMG-CoA) synthase and an HMG-CoA reductase (Figure 12.16) — toward mevalonate synthesis, which in turn activate the isoprenoid pathway for the synthesis of the sex and aggregation pheromones ipsdienol and ipsenol.

ALLELOCHEMICALS

The other major category of semiochemicals consists of the allelochemicals (Greek: *allelon*, of one another), which mediate interspecific interactions. These affect species other than the ones that are producing them and may adversely affect either the emitter or the receiver. If the signal is adaptively favorable to

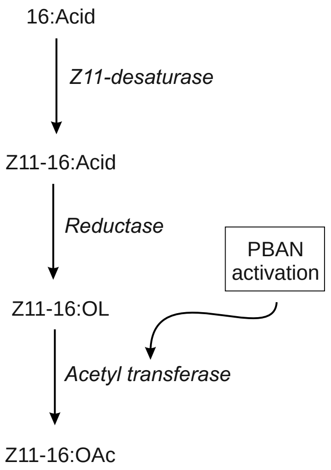

FIGURE 12.15. PBAN activates an acetyl transferase responsible for pheromone production in the moth, *Sesamia nonagrioides*. From Mas et al. (2000).

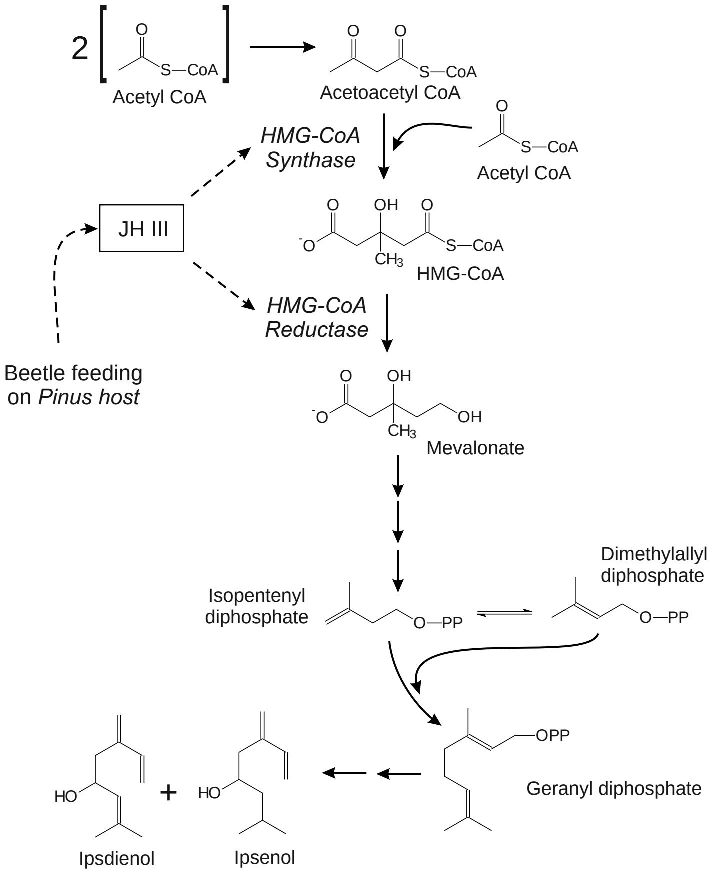

FIGURE 12.16. Pheromone biosynthesis in bark beetles and its control by JH. Two key enzymes involved in the pathway toward the synthesis of ipsenol and ipsdienol are activated by JH. From Tillman et al. (1999).

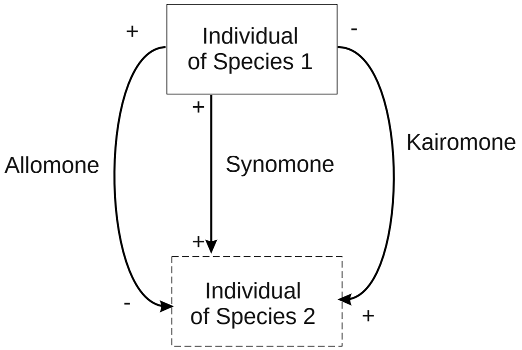

FIGURE 12.17. Allelochemicals, which mediate interspecific interactions. Allomones are allelochemicals that are adaptively favorable to the emitter but not the receiver. Kairomones are adaptively favorable to the receiver and not the emitter. Synomones are adaptively favorable to both the emitter and the receiver.

the emitter but not to the receiver, the substance is considered to be an **allomone**. If the signal is adaptively favorable to the receiver but not to the emitter, the substance is classified as a **kairomone**. If both the receiver and the emitter benefit, the substance is a **synomone** (Figure 12.17).

Allomones

Allomones are chemical countermeasures that are used primarily for defense. They are thus allelochemicals that are adaptive to the sender but not the receiver. A wide variety of chemical defenses can be mounted against potential predators, including oral and anal discharges, toxic components in the hemolymph made available by reflex bleeding, glandular discharges, and bites and stings that are supplemented with poisons.

Many orthopterans and the larvae of lepidopterans commonly discharge oral secretions consisting of gut contents mixed with salivary secretions when they are disturbed. In response to predation, some coleopterans engage in autohemorrhage, in which an allomone-fortified hemolymph that deters predators is released through the intersegmental membranes. Termite soldiers of some species supplement their large mandibles with toxic monoterpene hydrocarbons that are squirted on enemies as they fight. In the termite subfamily Nasutitermitinae, the soldier caste is composed of specialized **nasutes**, lacking mandibles for defense but capable of squirting a gummy latex substance from the frontal gland of their pointy heads (Figure 12.18). Bombardier beetles in the genus *Brachynus* discharge and accurately aim hot quinones at attackers. Synthesized as a binary weapon, hydrogen peroxide, hydroquinones, peroxidases, and catalases are separately

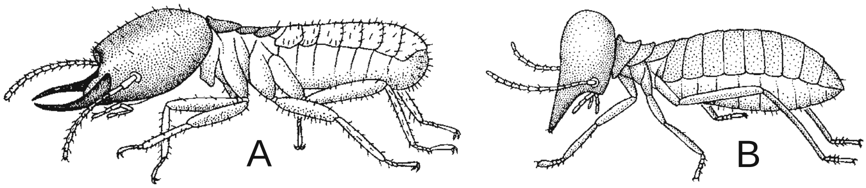

FIGURE 12.18. A. A typical member of the termite soldier caste of *Coptotermes*. B. A specialized nasute of *Nasutitermes*. From Gullan and Cranston (2000). Reprinted with permission.

stored in two chambers and mixed together in a reaction vessel to produce an explosive discharge at 100°C (Figure 12.19). Social Hymenoptera have modified their ovipositors into a sting apparatus that is used to effectively inject toxic and painful secretions under the skin of potential predators. Dytiscid beetles synthesize a mixture of steroids that are identical to the vertebrate hormones and discharge them into the water as a defense against vertebrate predators.

Given the extensive use of chemicals as pheromones to communicate between members of the same species, it is not surprising that some insects have evolved the role of illegitimate signalers that exploit these intraspecific chemicals and use them as allomones. Wild potato plants produce the alarm pheromone of the aphid *Myzus persicae*, (*E*)-β-farnesene, and prevent the aphids from feeding by releasing their alarm response. The flowers of orchid plants produce a number of volatiles that attract wasps for their pollination. The bolas spider produces a drop of moth sex pheromone at the end of a silken cord that it twirls around to entice the male moths to their deaths. A staphylinid beetle takes up residence in termite colonies and is able to acquire food from the termite workers because it has mimicked the cuticular hydrocarbons of the termite. In what has been called a "wolf-in-sheep's clothing" strategy, a *Chrysopa sloaaonae* lacewing larva plucks the wax from the wooly alder aphids on which it feeds and places the wax on its own dorsum. With this disguise, the lacewing is immune from assault by the ants that normally protect the aphids. This use of the cuticular hydrocarbon that establishes the identity of the aphids is unique as an allomone in that the lacewing does not synthesize it but physically coats itself with the secretions of its aphid prey.

Kairomones

Kairomones (Greek: *kairos*, opportunistic) benefit the receiver rather than the emitter. They have been described as pheromones and allomones that have evolutionarily backfired and therefore may not represent a distinct class of chemical signals themselves. Kairomones may be hormones, pheromones, or allomones that are normally used by one organism but exploited by an illegitimate receiver.

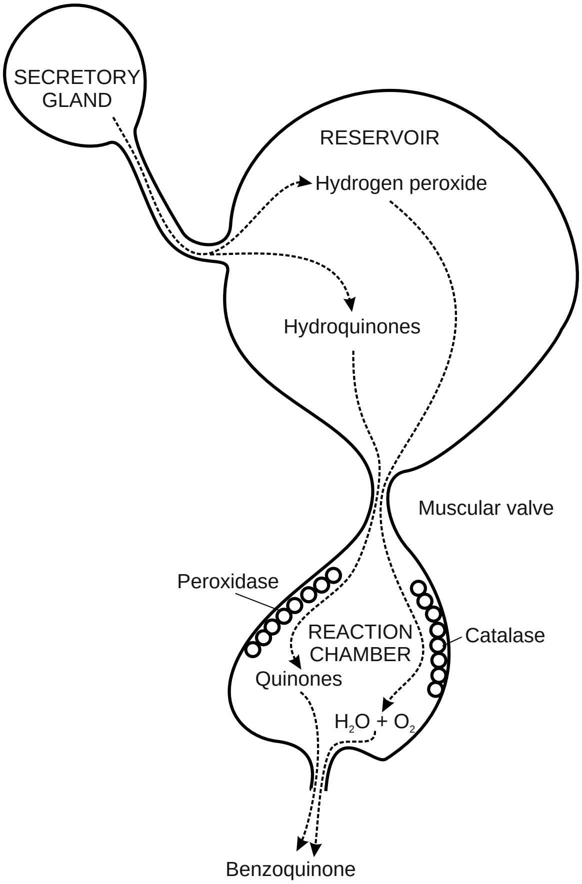

FIGURE 12.19. The production of hot benzoquinones by the bombardier beetle. The secretory gland produces hydrogen peroxide and hydroquinones into the reservoir. They are released into the reaction chamber, where the catalase and peroxidase reside, to produce an explosive reaction.

They may be normal products of metabolism of one species that another now uses to locate its host. For example, many phytophagous insects find their host plants by the chemical fingerprint of secondary plant substances that they use as kairomones. Mosquitoes locate vertebrate hosts for blood meals by using as kairomones the carbon dioxide and other chemicals that are produced during normal vertebrate metabolism. The parasitic mite, *Varroa jacobsoni*, is attracted to honeybee drone larvae by fatty acid esters present in the bee larvae, and the rabbit flea uses the hormones of its rabbit hosts as a kairomone to locate the rabbit and also to mature its reproductive system.

Illegitimate receivers have also evolved the ability to exploit the pheromones of other species and use them as kairomones. Predatory beetles and flies can recognize the aggregation pheromones of bark beetles to locate them as prey. A braconid parasitoid uses the epideictic pheromone produced by the apple maggot fly to find the eggs to parasitize. Some beetles, cockroaches, and mites can exploit trail pheromones that have been laid by foraging ants.

Synomones

Synomones are chemicals that are adaptive to both the sender and receiver. These include floral scents that attract pollinators and thus benefit both insect and plant. Hymenopterous parasites in the genus *Trichogramma* are attracted to tomato plants where they may find suitable hosts to parasitize. Damaged pine trees produce terpenes that bark beetles use as kairomones to find the trees, but the same chemicals are acting as synomones when they attract pteromalid hymenopterous parasites that then parasitize the beetles, benefiting both the parasitoids and the tree.

PRIMER PHEROMONES

The effects of pheromones acting as primers are at the same time subtle yet physiologically profound. They alter the physiology of the receiver so that it displays a modified response pattern to future stimuli. Unlike releasers, too few primer pheromones have been identified for them to be further subdivided categorically. Because the effects of primer pheromones are so subtle, possible bioassays for their actions are relatively difficult to perform and often take months to complete. The lack of suitable bioassays for primer pheromones has been an obstacle in their study and is certainly a major reason that the chemical nature of only one primer pheromone has been determined.

Primer pheromones are most often used by social insects for the regulation of colony activities. In honeybee colonies, there is only one fertile queen along with several thousand reproductively sterile workers. The queen produces a multicomponent pheromone from her paired mandibular glands, consisting of (E)-9-keto-2-decenoic acid, (R,E)-(–)- and (S,E)-(+)-9-hydroxy-2-decenoic acid, methyl *p*-hydroxybenzoate, and 4-hydroxy-3-methyoxyphenylethanol, collectively serving as the queen mandibular pheromone (QMP). QMP inhibits the ovarian development of workers and maintains them as nonreproductives, and it prevents the rearing of new queens. As workers age, there is a behavioral progression that reflects their changing roles within the colony. These changes in workers as a result of QMP exposure are reflected in many changes in gene expression in their brains. There are transient changes in the expression of a few

hundred genes as well as longer-term changes in a few others. Genes are activated and repressed that are correlated with the changing behavioral roles of workers; QMP activates genes correlated with nursing behavior and represses genes correlated with foraging behavior. Among its releaser functions, QMP serves as a queen recognition factor that is responsible for attracting and maintaining the retinue of workers that constantly attend to her and regulates the swarm behavior of the colony.

If the queen is removed, QMP levels decline within the colony, and the workers soon become agitated and begin preparations for rearing a replacement queen. They elongate existing cells that already contain newly hatched larvae and feed them royal jelly, a special secretion from their mandibular glands that, acting as a primer pheromone, brings the female larvae onto the developmental pathway to become queens rather than workers. The ingestion of royal jelly by the developing larvae affects their release of JH, and it is this presence of JH during a JH-sensitive period that ultimately determines which developmental polyphenism the larva will assume. Shortly after the last larval molt, larvae destined to be either workers or queens have identical ovaries, but during the next 24 h most of the ovarioles of the workers degenerate. The royal jelly also contains substances that preserve the food stored in the cells of the hive.

There are other primer pheromones in honeybees that originate with the brood. The larval cuticle of the brood contains a blend of 10 fatty acid esters and stimulates the development and synthesis of proteins by the hypopharyngeal glands of the workers.

The queen fire ant, *Solenopsis invicta*, produces a primer pheromone in its poison sac and possibly in another gland that has yet to be identified. Like the primer pheromone in honeybees, it prevents unmated queen ants that may be present in the colony from developing their ovaries and shedding their wings, and it acts as a releaser pheromone to attract workers to the queen. It acts indirectly on caste determination by affecting the behavior of the workers toward developing larvae. In response to primer pheromone, workers restrict the quantity of food given to female larvae that results in their development as workers.

There is also some evidence for primer pheromones in nonsocial insects. Adult male *Schistocerca gregaria* locusts produce a primer pheromone on the surface of the cuticle that accelerates the growth of male and female immatures.

THE MULTICOMPONENT NATURE OF BEE COMMUNICATION

Humans have been aware of the ability of bees to communicate the location of resources to others in the hive at least since Aristotle wrote about the phenomenon of honeybee recruitment more than 2000 years ago. The method by which

this communication occurs was first discovered by Von Frisch, who described how a foraging worker returns to the hive and communicates both the distance and the direction of the resources by performing a dance for other workers using a symbolic language (Figure 12.20). If the food is close to the hive, the returning forager performs a round dance that gives no information about the direction of the food, but indicates only that the food is nearby. If the food is distant, the forager performs a waggle dance that imparts both direction and distance. The forager performs the waggle dance on the vertical surface of the combs within the hive, based on the distance of the food and its angular direction with respect to the sun. The nervous system of the bees is able to compensate for the movement of the sun during the intervening period. Accompanying the dance are sounds produced by both the forager and the other workers in response. The forager produces an airborne sound by beating her wings as she dances, and an observing worker responds by producing sounds as she presses her thorax against the comb. These comb vibrations are cues the forager uses to stop dancing and

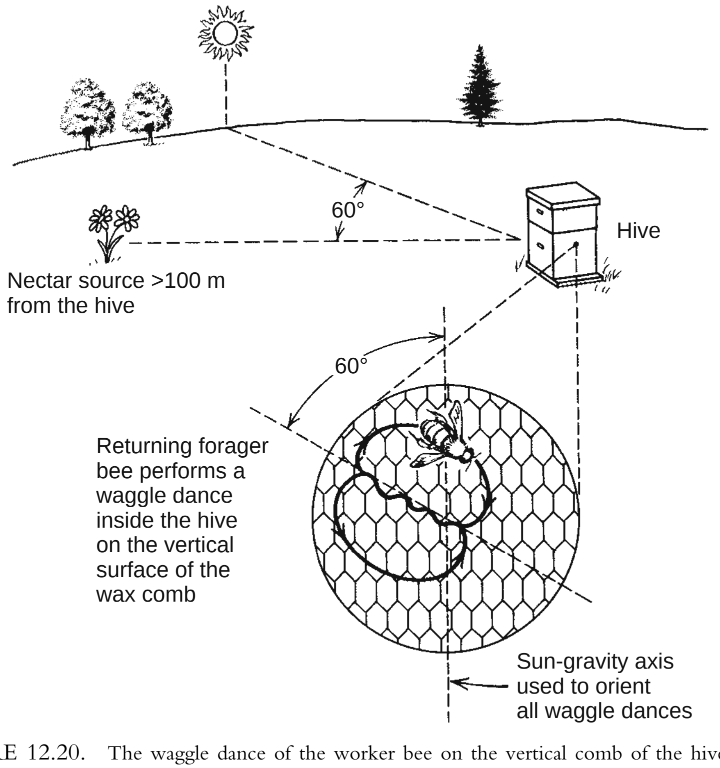

FIGURE 12.20. The waggle dance of the worker bee on the vertical comb of the hive is performed at an angle equal to the angle of the nectar source from the hive. From Matthews and Matthews (1978). Reprinted with permission.

regurgitate food samples to give the workers additional information about the quality of the food source. Most races of honeybees have their own dialects of the dance language that may reflect the differences in the ecological ranges in which they forage, but they all communicate with vision, sound, and tactile stimuli.

The dance language represents a relatively small proportion of the communication that occurs in a functioning colony of honeybees. Colony members communicate using an extensive array of chemical signals in addition to the QMP and alarm pheromones described in earlier sections. The Nasanov pheromone, a seven-component blend released from worker abdomens, attracts other workers in a nonaggressive manner during swarming. Developing larvae produce a mixture of ethyl and methyl esters that inform the workers to cap the cell before their pupation. In honeybee society, the language of communication is based on nearly 50 substances that are essential for its proper functioning.

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Glossary

A band The dark band within a muscle sarcomere that is formed from the actin proteins.

Abductor muscle A muscle that moves the distal portion of an appendage away from the body.

Accessory gland Glands that are part of the male and female reproductive systems. In the female, they secrete cement, venoms, and lubricants. In the male, they produce the seminal fluid, spermatophore, and active peptides that affect the female.

Accessory pulsatile organ An accessory heart at the base of the wings, legs, and antennae that supplement the movement of hemolymph from the body cavity.

Acetylcholine A neurotransmitter that is released from the presynaptic neuron and binds to receptors on the post-synaptic neuron of the synapse to perpetuate the action potential across neurons.

Acetylcholinesterase The enzyme present in the synapse that breaks down acetylcholine to acetate and choline, making the acetylcholine receptors available for the next release of neurotransmitter.

Acrosome The organelle at the tip of the sperm that is derived from the Golgi apparatus and breaks down the egg membrane to allow the sperm to penetrate for fertilization.

Actin A protein that makes up the muscle myofibrils. Together with myosin, it mediates muscle contraction.

Action potential A change of constant amplitude in the membrane potential of a neuron that is triggered by a depolarization of the dendrite. The action potential sweeps down the axon to the synapse.

Acyl carrier protein A protein linked to intermediates in the synthesis of fatty acids.

Adipohemocyte A type of insect hemocyte characterized by a relatively small nucleus and a cytoplasm containing abundant fat globules.

Adipokinetic hormone A hormone often produced by the corporus cardiacum that acts on the fat body to cause it to release stored lipids into the hemolymph.

Aedeagus The intromittent organ of copulation in the male.

Aeropyle A modification of the egg chorion that permits gaseous exchange.

Aggressive mimicry The behavior of a prey species when it mimics some aspect of its prey to facilitate capture.

Alarm pheromone A chemical produced by members of a species that induces a behavioral alarm or alertness in other individuals of that species.

Alary muscle Paired muscles that support the heart within the body cavity.

Allatostatin A hormone produced by the brain that inhibits juvenile hormone production by the corpus allatum.

Allatotropin A hormone produced by the brain that stimulates juvenile hormone production by the corpus allatum.

Allele An alternative variant of a given gene.

Allozyme A variant of an enzyme that is encoded by a different allele.

Ametabolous A type of development without any metamorphosis. Found in apterygotes where, except for size, adults and immatures closely resemble each other and have similar ecological habits.

Amniotic cavity A cavity formed by the amniotic folds during development. It encloses the germ band and frees it from the rest of the embryonic serosa.

Amplification An increase in the number of additional copies of a specific DNA sequence.

Anabolism The metabolic production of protein, carbohydrates, and fats from ingested food.

Anthropomorphism The tendency to attribute human characteristics to non-human animals.

Apneustic A tracheal system that lacks spiracles.

Apolysis The separation of the epidermis from the overlying cuticle. Apolysis marks the beginning of the molt.

Apomictic parthenogenesis A form of reproduction in which the oocyte does not undergo any reduction division but remains diploid and the embryo develops as a clone of the mother.

Apoptosis The programmed death of a cell.

Apposition eye A type of compound eye used by day-active insects in which each ommatidium is surrounded by pigment cells and isolated from the light that enters neighboring ommatidia.

Apyrene sperm Sperm, mostly produced by male lepidopterans, that lack a nucleus.

Archicerebrum The primitive ganglionic mass that gave rise to the brain.

Arrhenotoky A type of parthenogenesis that results in the production of only male offspring.

Arthrodial membrane The intersegmental membrane between two sclerotized plates that bestows flexibility to the cuticle. The arthrodial membrane typically consists of endocuticle covered by epicuticle, with little exocuticle.

Asynchronous muscle A specialized flight muscle that only requires periodic activation by the central nervous system. Once activated, the stretching by antagonistic muscles stimulates its contraction.

ATP Adenosine triphosphate, the major molecule used for storing and transferring chemical energy in a cell.

Atrium The chamber immediately below the spiracle.

Autogeny The ability to produce eggs without requiring a reproductive meal.

Automictic parthenogenesis A type of reproduction that does not require fertilization by sperm. The egg is haploid but the diploid number is restored by fusion of the egg nucleus with a polar body.

Axoneme The organelle that propels the sperm by causing the flagellum to move. The axoneme consists of a series of microtubules.

Basement membrane The innermost layer of the integument that is secreted by hemocytes, forming a continuous layer of connective tissue that separates the body cavity from the integument.

Bivoltine Insect species with two generations per year.

Blastoderm A continuous layer of cells that surrounds the egg early during embryogenesis, derived from the energids that migrate to the periphery.

Blastokinesis A displacement of the embryo within the egg that enables it to utilize more yolk.

Bombyxin An insulin-like peptide that was first characterized as a "small" PTTH, but is not believed to have PTTH activity. Among several other possible functions, bombyxin regulates hemolymph carbohydrate levels.

Brachial chamber A region within the rectum of larval dragonflies that contains tracheal gills that can extract oxygen from the water taken up through the anus.

Buccal cavity The region of the alimentary canal surrounded by the mouthparts and anterior to the true mouth.

Bursa copulatrix A modified pouch in the genital tract of some female insects into which sperm are first deposited before they migrate to the spermatheca.

Bursicon A hormone that mediates cuticular sclerotization.

Calyx The pedicel of each ovariole that leads to the lateral oviduct.

Campaniform sensillum A dome-shaped sensory receptor that measures cuticular distortion.

Cardioacceleratory peptide A peptide that regulates the rate of contraction of the dorsal vessel.

Cardioblast Embryonic cells that develop into parts of the dorsal vessel.

Catabolism The metabolic processes that break down ingested organic molecules and release energy, captured by ATP, and waste products.

Cecropin A family of hemolymph proteins that mediate a humoral response to foreign invaders.

Cement layer The outermost layer of the epicuticle, produced by dermal glands and consisting of a shellac-like coating. The cement layer seals the wax layer and provides protection from physical abrasion.

Cerebral ganglion The consolidated ganglia of the head that form the brain.

Chitin A polysaccharide consisting of linked *N*-acetyl-glucosamine residues. Chitin is insoluble in water, dilute alkali, alcohol, and organic solvents, but it is soluble in concentrated acids and hot alkali solutions. The chains of chitin are associated with protein, providing a framework for the stabilization of the cuticular proteins.

Chitosan Chitin treated with concentrated alkali at high temperatures is

deacetylated to yield chitosan, which produces a characteristic violet color with iodine as an indication that chitin was once present.

Chordotonal organ A subcuticular sensillum made up of units called scolopidia, attached to the cuticle at one or both ends. External movement distorts the scolopidium and causes the dendrite to depolarize.

Choriogenesis The synthesis and production of the chorion, or eggshell.

Chorion The complete outer shell of the insect egg. Its synthesis is one of the last acts of the follicular epithelium that surrounds the oocyte before it degenerates.

Chromosome puff Areas on the chromosome visible under light microscopy that are evidence of mRNA transcription.

Cibarium The area within the pre-oral cavity where food remains before it enters the mouth and the true digestive tract.

Circumesophageal connectives Nervous connections between the brain and the ventral nerve cord.

Citric acid cycle A series of aerobic biochemical reactions that ultimately degrade carbohydrates, fatty acids, and proteins to carbon dioxide and water and capture the energy differences in ATP.

Cleavage A series of mitotic divisions that transform the zygote into an embryo.

Colleterial gland The female accessory gland that produces a cement used to attach eggs to the substrate.

Columnar cells The most common cell type in the insect midgut, with a highly convoluted microvillar membrane facing the gut lumen.

Compound eye An optical receptor that is comprised of many individual, optically sensitive units, or ommatidia.

Corneagen cell Modified epidermal cells that produce the cornea of the compound eye.

Corneal lens The lens of the compound eye.

Corpora allata Ductless glands, usually found in pairs (singular: corpus allatum), that synthesize and release juvenile hormone.

Corpora cardiaca The neurohemal organ for the neurosecretory cells of the brain, also containing secretory cells of its own. The usually paired structures (singular: corpus cardiacum) store and release a number of neurohormones synthesized in the brain.

Corpora pendunculata The mushroom bodies of the protocerebral lobes of the brain. They contain abundant nerve cell perikarya and interneurons. Its size is correlated with behavioral complexity and is most highly developed in social Hymenoptera that display complex behaviors.

Countercurrent heat exchange The thermoregulatory mechanism that maintains a warm thorax. Cooler hemolymph from the abdomen is moved to the thorax at the same time that warm hemolymph from the thorax is moving backward. The warm hemolymph is used to preheat the cooler hemolymph before it enters the thorax.

Cryptonephridium A modification of the excretory system in insects that live in dry habitats. The distal ends of the Malpighian tubules are directly in contact with the rectum and remove water and salts before they are excreted.

Crystalline cone A hard transparent refractive structure of the compound eye.

Cytochrome Mitochondrial respiratory enzymes that function as electron carriers in biological oxidation.

Cutaneous respiration The ability to take up oxygen through the integument.

Cuticulin A layer of the epicuticular envelope. Also called the outer epicuticle, the epicuticle is deposited on its inner surface.

Cystocyte A cell that arises from the division of a cystoblast originating from stem cells.

Defensin A peptide that mediates humoral immunity.

Dermal gland A modified epidermal cell that produces the cement layer as well as defensive secretions and pheromones.

Determination The nonvisible commitment of an undifferentiated cell to its differentiated state.

Deuterotoky A type of parthenogenesis that can result in either male or female offspring.

Deutocerebrum The middle portion of the insect brain that produces neurons that innervate the antennae.

Differentiation The visible physiological commitment of a cell to a specific developmental pathway.

Diploid An organism with two copies of each chromosome.

Direct flight muscles Thoracic muscles that connect directly with the wing.

Diuretic hormone A hormone that increases the activity of the Malpighian tubules.

Diverticulum An invagination of the alimentary canal that produces a blind sac for the storage of ingested food.

Dorsal closure A stage of embryogenesis in which the embryonic ectoderm grows over the dorsal side of the embryo.

Dorsal diaphragm Connective tissue and muscle that form a dorsal compartment that separates the perivisceral and pericardial sinuses.

Dorsal ocelli The simple eyes usually found on the vertex of the head between the compound eyes.

Dorsal vessel The structure used for pumping hemolymph forward from the abdomen into the head. The dorsal vessel is divided into the posterior heart and anterior aorta.

Dyar's rule A generalization from the work of Harrison Dyar (1890) that predicted a geometrical increase in the head capsule width of lepidopteran larvae that could be used to predict the instar number.

Dynein A contractile protein found in the cilia and flagella. Dynein is a component of the flagella of insect sperm.

Ecdysial line A programmed area of weakness in the cuticle that allows the insect to emerge when the endocuticle is sufficiently digested.

Ecdysiotropin A hormone that stimulates the production of ecdysteroids.

Ecdysis The process of shedding the old cuticle at the end of molting.

Ecdysis triggering hormone A hormone released from epitracheal glands that acts on the cells of the ventral nerve cord and causes the initial expression of preecdysis behavior and the release of eclosion hormone.

Ecdysteroid The collective term for derivatives of ecdysone. Ecdysteroids trigger mainly apolysis and vitellogenin production by affecting gene expression.

Eclosion The ecdysis of adult insects and the hatching of first instar larvae from the egg.

Eclosion hormone A peptide that initiates the stereotyped behaviors associated with ecdysis.

Ectoderm The outer germ layer of the embryo.

Ectoperiptrophic space The compartment created by the peritrophic membrane in the midgut.

Ectothermy Body temperature regulation using external sources, usually the sun.

Ejaculatory duct The common ectodermal duct uniting the vasa deferentia of the male reproductive system. Sperm travel from the vas deferens to the ejaculatory duct and into the female.

Electrophoresis The separation of molecules by their electric charges in an electric field.

Embryogenesis The period of development that begins within the egg with germ band formation and ends with dorsal closure of the embryo.

Embryonic primordium The thickened portion of the blastoderm that will develop into the embryo.

Endochorion A middle layer of the chorion that can be divided into an inner endochorion with a network of pillars, and the outer endochorion that creates a roof network.

Endocrine gland A gland that produces and secretes hormones within the body.

Endocuticle The innermost layer of the cuticle secreted by epidermal cells. It is unsclerotized and capable of being resorbed during the molting process.

Endoderm The inner germ layer of the embryo that gives rise to the midgut.

Endonuclease An enzyme that degrades nucleic acids by the internal cleavage of phosphodiester bonds.

Endoperitrophic space A space within the gut that is created by the peritrophic membrane.

Endoplasmic reticulum (ER) An organelle consisting of membrane-bound cavities within the cytoplasm that are continuous with the plasma membrane of the cell. Rough ER is coated with ribosomes; smooth ER is free of ribosomes.

Endopodite The middle branch of a biramous appendage.

Endopterygote Insects that undergo a metamorphosis with their wings developing on the inside of the body.

Endoskeleton An invagination of the body wall that provides rigidity and attachment sites for muscles.

Endothermy The use of heat generated from flight muscles to warm the body.

Energids The daughter nuclei from the mitotic division of zygote nucleus during meroblastic cleavage, surrounded by an island of cytoplasm.

Epicuticle The thin, top layer of the cuticle, consisting of the inner and outer epicuticles, the wax layer, and the cement layer.

Epideictic pheromone A chemical substance produced by one individual that maintains the proper density among other individuals of that species.

Epidermis The single layer of cells that secretes the cuticle.

Epitracheal gland A group of neurosecretory cells associated with the trachea that produce the ecdysis-triggering hormone.

Esophagus A portion of the foregut that is differentiated as a simple tube that leads to the crop.

Esterase An enzyme that hydrolyzes esters.

Eupyrene sperm A type of sperm in lepidopterans that contains a nucleus and is used to fertilize eggs.

Exochorion The proteinaceous outermost layer of the chorion.

Exocrine gland A gland that secretes chemicals to the outside of the body.

Exocuticle The outer layer of the procuticle that is sclerotized and incapable of resorption.

Exopterygote Insects that undergo a metamorphosis in which the wings develop as buds on the outside of the body wall.

Exoskeleton The hardened body wall of an insect.

Exuviae The old skin that is cast during the molting process. Always used as a plural noun.

Exuvial space The area created between the epidermal cells and the old cuticle by the digestion of the old endocuticle during the molting process.

Fate map A map of precise sites on the embryonic blastoderm that corresponds with anatomical structures later in development.

Fast neuron A type of neuron that releases a large number of neurotransmitter packets to cause a strong muscle contraction.

Fertilization The union of the haploid egg and sperm to produce a diploid zygote.

Fixed action pattern An innate stereotyped motor program.

Follicular epithelium The cells that surround the oocyte, sequester yolk proteins, and synthesize the chorion.

Foregut The first of the three principal divisions of the insect alimentary canal. The foregut begins at the mouth and continues to the proventriculus.

Fovea A group of ommatidia in the center region of the compound eyes that are capable of higher spatial resolution.

Freeze avoiding Insects that produce hemolymph cryoprotectants that allow them to supercool to as low as –35°C and remain in a liquid state without the formation of ice crystals.

Freeze tolerant Insect's that are able to survive the formation of extracellular ice crystals by synthesizing ice-nucleating proteins that raise the supercooling point of body fluids and serve as catalysts for the extracellular nucleation of ice.

G-proteins A family of signal-coupling proteins that act as intermediaries between activated membrane receptors and cellular effectors.

Gamete A germ cell; the sperm or egg.

Gap genes Genes that are expressed in broad domains of several body segments of the embryo. Mutants cause gaps in the developmental pattern.

Gastric caecae Saclike diverticulae at the anterior region of the midgut that are involved with enzyme secretion and ion movements.

Gastrulation During embryonic development, the formation of the gastrula from endoderm.

Gene duplication The duplication of a DNA segment that codes for a specific gene, allowing the organism to produce much larger amounts of a specific protein.

Genome All of the genetic information that an organism possesses.

Germ band The region of thickened blastoderm cells that becomes the embryo later in development.

Germarium The anterior regions of the male and female reproductive systems that produce oocytes from oogonia and spermatocytes from spermatogonia.

Glial cell An accessory cell that surrounds neurons and provides them with nourishment and insulation.

Glycerol-3-phosphate shuttle A biochemical pathway that regenerates NAD^+ from NADH by transferring hydrogen atoms between the cytoplasm and mitochondria.

Glycogen A branched-chain polysaccharide used by animals for storing carbohydrate.

Gnathal segments The body segments that ultimately bear the appendages that become the mouthparts.

Goblet cell A cell present in the midgut of larval lepidopterans that secretes potassium into the lumen and creates a flow of water in the gut.

H zone The area visible in the center of the A band, consisting of myosin filaments without any overlapping actin.

Haploid Cells that contain a single copy of each chromosome.

Haploid parthenogenesis A type of reproduction in which the oocyte divides meiotically to form a haploid gamete that may develop with or without fertilization.

Hemimetabolous Insects that have a gradual or incomplete metamorphosis and the development of wings on the outside of the body wall.

Hemocele The body cavity containing the internal organs and hemolymph.

Hemocyte One of a variety of different blood cells of insects.

Heterogametic sex The sex that produces gametes that may contain different sex chromosomes. Male insects are usually heterogametic, producing either X or Y chromosomes in their gametes.

Hindgut The posterior region of the alimentary canal derived from the proctodeal invagination.

Histoblast A small group of cells that give rise to a particular tissue.

Holoblastic A type of cleavage during embryogenesis in which the entire egg cell is divided.

Holometabolous The type of metamorphosis in which a complex change occurs between larvae and adults, involving a pupal stage. Wings develop on the outside of the body wall.

Holopneustic The condition of the respiratory system in which all spiracles are open and functional, usually involving two thoracic and eight abdominal spiracles.

Homeobox A conserved DNA segment of 180 genes that is present in all homeotic genes.

Homeotic genes Genes that specify the identity of body segments during embryogenesis.

Homeotic mutants Mutations in which one body part is replaced with another homologous one.

Homologous Structures that have a common evolutionary origin.

Homozygous Diploid cells that contain two identical alleles of a particular gene.

Hormone A chemical produced by specialized tissues and released into the blood that affects target tissues elsewhere in the body.

Hypermetamorphosis Endoptyergote insects whose larvae change form before pupation, producing different forms during the larval stage.

Hypocerebral ganglion A part of the stomodeal nervous system; a group of neurons that begins at the frontal ganglion and continues rearward to innervate the gut.

Hypopharynx A tonguelike structure that develops as a internal lobe of the head and often divides the pre-oral cavity into a cibarium and a salivarium.

I band The muscle band seen in longitudinal section that consists of only the actin filaments.

Imaginal disc A group of cells that is sequestered during larval development and gives rise to adult organs during the pupal stage.

Indirect flight muscles The dorsoventral and dorsal longitudinal muscles in the thorax that change the conformation of the thorax and indirectly move the wings.

Inner chorionic layer A thin proteinaceous inner layer of the chorion that is secreted above the vitelline envelope.

Integument The components of the exoskeleton, consisting of the basement membrane, epidermis, and cuticle.

Interneuron A neuron that lies entirely within a ganglion and serves as a switch to direct the stimuli from other neurons.

Intron A region of a eukaryotic DNA that codes for an RNA sequence that is removed during later processing.

Ion channels Passages in a cell membrane that allow certain ions to pass into or out of the cell.

Isozyme A different form of the same multisubunit enzyme. In contrast to allozymes, isozymes are the result of differing subunit configurations rather than allelic differences.

JH binding protein A protein that binds to JH and protects it from degradation while circulating in the hemolymph.

JH response element A short, specific sequence of DNA located in the promoters of JH-responsive genes.

Johnston's organ A sensillum found in the second antennal segment that monitors antennal deflection.

Juvenile hormone (JH) A sesquiterpene that is produced by the corpus allatum and has a wide range of effects on metamorphosis and reproduction.

Kairomone An interspecific chemical message that benefits the receiver but not the emitter.

Kinesis A nondirectional response of an organism to a stimulus.

Laminar A composite structure consisting of several flat layers.

Lateral ocelli The simple eyes found in holometabolous larvae.

Lateral oviduct The tube that extends from the calyx of the ovary to the median oviduct, usually derived from embryonic mesoderm.

Lipophorin A protein carrier that binds to lipid molecules in the hemolymph,

transporting them from the fat body to target tissues.

Locus The position of a gene on the chromosome.

Long germ band Embryonic development where the pattern of segmentation is established by the end of the blastoderm stage.

Luciferase The enzyme that acts on the substrate luciferin in the presence of oxygen and ATP to produce light.

Luciferin The molecule that is oxidized by luciferase to produce light.

Malpighian tubules The excretory organs of insects, arising at the junction of the midgut and hindgut.

Median oviduct The tube that connects the lateral oviducts and expels eggs out of the body of the female. The median oviduct is derived from ectodermal invaginations and may be modified into a genital pouch for the incubation of eggs.

Meiosis The process of cell division that results in haploid germ cells from a diploid cell.

Melanin A dark, organic pigment deposited by the epidermal cells.

Meroblastic A type of embryonic cleavage that divides only the nucleus but not the cytoplasm, ultimately producing a synctium of blastodermal cells.

Meroistic The type of ovariole that contains trophocytes, or nurse cells, to nourish the oocyte.

Mesocuticle A transitional layer of the cuticle between the endocuticle and exocuticle.

Mesoderm The middle embryonic layer of tissue that is derived from the interaction between the ectoderm and the endoderm.

Metabolism The process by which food is transformed into body tissues and waste products.

Metapneustic The type of respiratory system in which only the spiracles on the last abdominal segment are functional.

Methoprene A juvenile hormone mimic that is used as an insect growth regulator.

Micropyle A pore in the chorion of the egg through which sperm can enter for fertilization.

Midgut The central portion of the alimentary canal between the foregut and hindgut where most digestion occurs. The midgut is derived from endodermal tissues.

Mitosis The division of a single cell into two daughter cells.

Molting gel An enzyme produced by epidermal cells that digests the old endocuticle during the molting process. It is first secreted in an inactive form and is activated once the new cuticle begins to be formed.

Morphogen A substance that influences the movement and gene transcription of cells through a concentration gradient.

Motor neuron A neuron that innervates muscles.

Muscle fiber A muscle cell, often multinucleate and containing myofibrils within its cytoplasm.

Mushroom body An anatomical region of the pars intercebralis of the brain that is dense with synaptic contacts.

Myofibril Contractile proteins embedded in the cytoplasm of muscle fibers that consist of the myofilaments, myosin, and actin.

Myofilament The individual myosin or actin molecules that constitute the myofilaments in muscle.

Myogenic Muscle contractions that occur independently of nervous stimulation.

Myosin A large globular protein that comprises the thick myofilaments of muscle cells.

Myotropin A peptide that causes muscle contraction.

Nanogram One billionth of a gram.

Nephrocyte Cells found mostly in the pericardial sinus that take up nonparticulate colloids and release metabolites into the hemolymph.

Neural superposition eye A type of compound eye that is optically appositional, but whose neurons are configured so that light from neighboring ommatidia make it functionally superpositional.

Neuroblast Cells that give rise to neurons.

Neurohormone A hormone produced by neurosecretory cells and released from a neurohemal organ into the hemolymph.

Neuromodulator A neurotransmitter released at the synapse that modifies the conditions under which other nerve impulses are transmitted and received.

Neuropil The mass of neurons within a ganglion.

Neuropeptide Short-chain peptides produced by neurosecretory cells.

Neurosecretory cell A specialized neuron that produces hormones that are released into the hemocoel.

Neurotransmitter A chemical released at the neural synapse that enables the nervous activation to pass to an adjacent neuron.

Nidi Groups of regenerative cells in the midgut (singular, nidus).

Nodule A mechanism of cellular defense in which small foreign objects are surrounded by hemocytes and sequestered.

Odorant binding protein A peptide in the receptor lymph that binds to odorant molecules that enter through the pore tubule and shuttles the molecules to receptor sites.

Oenocyte A specialized hemocyte that usually resides between the basement membrane and the epidermal cells. Oenocytes appear to synthesize wax and possibly ecdysteroids.

Oenocytoid A hemocyte that resembles an oenocyte.

Oocyte The female gamete that differentiates from oogonia.

Oogonia The first stage in the differentiation of an oocyte from a primary female germ cell.

Ootheca The protein synthesized to cover an egg mass.

Operculum An area of programmed weakness on the insect chorion that allows the first instar larva to escape from the egg.

Opsinization The coating of a foreign particle used to assist in its phagocytic ingestion.

Optic lobes The lateral lobes of the protocerebrum that supply nerves to the compound eyes.

Osmosis The diffusion of a solvent, usually water, from a dilute solution to a more concentrated solution through a semipermeable membrane.

Ostia The slitlike valves in the heart that allow hemolymph to enter (singular: ostium).

Ovariole The tubes that compose the ovary and contain the oocytes as they develop into eggs.

Ovary The paired female reproductive organs.

Oviparity The most common form of insect development, in which the egg is fertilized as it is oviposited and develops outside the female's body.

Oviposition The passage of the egg to the outside of the female.

Ovoviparity A specialized form of development in which the egg is retained and incubated within the common oviduct. The egg hatches shortly after it is oviposited.

Ovulation The passage of the egg out of the ovariole and into the lateral oviduct.

Oxidation The energy-releasing process of removing electrons from a substrate.

Paedogenesis The specialized form of reproduction in which the ovaries become mature during the immature stages and the larva produces eggs that develop parthenogenetically.

Pair rule genes Genes that are expressed in stripes along the embryonic blastoderm in a periodicity that corresponds to every other parasegment.

Panoistic An ovariole that lacks nurse cells and nourishes the oocyte through the follicular epithelium.

Parasegment Visible cuticular segments during development that are controlled by the pair rule genes but that do not represent the true embryologic segments.

Pars intercerebralis The medial portion of the protocerebrum of the brain that often contains neurosecretory cell bodies.

Parthenogenesis Egg development that occurs without fertilization by the male gamete.

Patency The opening between follicle cells that permits the uptake of vitellogenin from the hemolymph.

PBAN Pheromone biosynthesis activating neuropeptide, responsible for the activation of enzymes involved in pheromone synthesis.

Pericardial cell Specialized cells that lie at the side of the heart and filter the blood.

Pericardial sinus The sinus formed by the dorsal diaphragm that consists of the hemolymph cavity around the heart.

Perikaryon A nerve cell body that contains the nucleus.

Perineural sinus The sinus formed by the ventral diaphragm that consists of the hemolymph cavity around the ventral nerve cord.

Perineurium A sheath that surrounds a group of neurons.

Peripheral nervous system The system of nerves consisting largely of sensory receptors.

Periplasm The region of the oocyte cytoplasm that lies just below the vitelline membrane.

Peritracheal glands Small groups of neurosecretory cells located around the tracheae.

Peritreme The cuticular sclerite that surrounds a spiracle.

Peritrophic matrix A delicate chitinous matrix secreted by midgut cells that protects the cells and forms digestive compartments within the gut.

Perivisceral sinus The sinus formed by the dorsal and ventral diaphragms that

consists of the hemolymph cavity around the digestive tract.

Phagocyte A blood cell that engulfs and consumes foreign bodies in the hemolymph.

Pharate The state of the instar after apolysis but before ecdysis; the instar concealed by the old cuticle.

Phasic response A response of a neuron or whole sensillum in which an action potential is generated only when the sensillum is distorted.

Pheromone A chemical produced by an individual of one species that mediates behavior of another individual of the same species.

Phragma An epidermal invagination producing an internal ridge for muscle attachment.

Phylogeny The evolutionary history of an organism.

Phytoecdysteroid Ecdysteroids that are produced and derived from plants.

Plasma The noncellular liquid portion of the hemolymph.

Plasmatocyte The basic form of insect hemocyte; large, abundant insect blood cells.

Plastron A physical gill found in aquatic insects in which a bubble of air is held in place by hydrofuge hairs and allows oxygen to be extracted from the water.

Plectrum A part of a stridulatory apparatus consisting of a structure that is rubbed against a membrane.

Pole cells Energids that migrate to the posterior pole of the egg early during development and ultimately differentiate into the germ cells of the adult.

Polyacrylamide gel electrophoresis A method of separating molecules by their size and charge.

Polyembryony The production of several embryos from a single egg, a form of reproduction occurring in some parasitic hymenopterans and Strepsiptera.

Polyphenism The presence of several different phenotypes in a population that are determined by environmental factors and not genotype.

Polytene chromosome Chromosomes that have undergone duplication without separating.

Polytrophic meroistic A type of ovariole in which nurse cells accompany the oocytes within the follicle and supply it with nutrients.

Pore canal Small channels that extend from epidermal cells through the cuticle up to the epicuticle and carry substances such as waxes.

Preoral cavity The space enclosed by the mouthparts, forming a cavity in front of the true mouth, which is the beginning of the alimentary canal.

Primary sense cell A sensillum that is directly innervated by the nervous system without synapsing first with an interneuron.

Primary urine The waste products produced by the Malpighian tubules and emptied into the hindgut. The primary urine is modified by the rectum and excreted as secondary urine.

Primer pheromone A chemical produced by an individual of one species that has a fundamental physiological effect on another individual of the same species.

Prizbram's rule A generalization regarding insect growth, assuming that body weight doubles for each larval molt as a consequence of the ratio of surface area to body volume.

Proctodeum The tissue formed by the epidermal invagination at the posterior end of the embryo that produces the hindgut.

Procuticle The undifferentiated chitinous cuticle that develops into the endocuticle and exocuticle.

Prohemocyte Small, round hemocytes that give rise to other hemocyte types.

Promoter A region of DNA necessary to initiate gene transcription.

Pronymph A nonfeeding stage in some hemimetabolous insects that have recently hatched; it is less developed than the later nymphal stage. Shifts in the temporal production of juvenile hormone may have led to the evolution of a larval stage from this pronymph stage.

Propneustic The configuration of the tracheal system in which only the anterior spiracles are open and functional.

Proprioreception The perception of where an organism's body parts are immediately located.

Prothoracic gland The endocrine gland that synthesizes and secretes ecdysteroids.

Prothoracicotropic hormone The hormone produced by neurosecretory cells in the brain that activate the prothoracic gland to synthesize ecdysteroids.

Protocerebrum The anterior and most complex lobe of the brain, subdivided into protocerebral and optic lobes.

Proventriculus A specialized area of the posterior region of the crop that serves as a valve for the passage of food into the midgut and can be modified into a gizzard for grinding food before its digestion.

Puffing A swelling of localized areas on the chromosome that indicate gene transcription.

Receptor potential The change in the membrane potential of a sensillum after stimulation. The potential change is proportional to the strength of the stimulus.

Rectal pad Specialized cells in the rectum involved in the uptake of materials from the lumen to the hemolymph.

Rectum The posterior portion of the hindgut, modified to resorb fluids from the lumen and return some of the needed components to the hemolymph.

Reduction The energy-storing process that involves the addition of electrons to a substance.

Reflexive bleeding The defensive release of hemolymph that contains distasteful substances through intersegmental membranes to avoid predation.

Releaser pheromone A chemical released by an individual of one species that has an immediate effect on releasing some behavior of other individuals of that same species.

Resilin A colorless rubbery cuticular protein that confers elasticity to areas of the cuticle that contain it. Resilin is capable of storing and releasing mechanical energy.

Resting potential The normal, steady membrane potential in an unstimulated neuron.

Retinula cell A cell comprising the ommatidia of the compound eye that contains optically active pigments and is stimulated upon the reception of light.

Reynolds number A number that expresses the relationship between the

size of an organism and the physical forces acting upon it. The Reynolds number is a ratio of inertial and frictional forces.

Rhabdom The optically active structure of a light receptive sensillum, consisting of the individual rhabdomeres of adjacent retinula cells.

Rhodopsin A protein pigment capable of absorbing a photon of light and transferring the energy to other biological molecules.

Ring gland The composite endocrine gland of larval dipterans consisting of the prothoracic gland, corpus allatum, and corpus cardiacum.

Salivarium The posterior portion of the pre-oral cavity into which the salivary duct empties.

Sarcolemma The cell membrane of a muscle cell.

Sarcomere A unit of muscular contraction between the two Z lines, containing the myofibrils.

Sarcoplasm The cytoplasm of a muscle cell.

Scolopale A rodlike capsule that covers the distal end of the dendrite in a chordotonal organ.

Second messenger A substance that acts inside the cell to alter the rate of biological processes once it is activated by a hormone that acts on the outside of the cell.

Secondary urine The urine after it is processed by the rectum and eliminated from the body.

Segment polarity gene Developmental genes that determine a linear sequence of positional values within each segment.

Seminal fluid The product of the male accessory glands that serves as a transport medium for sperm and contains physiologically active substances that can affect the physiology of the female that receives it.

Seminal vesicle The enlarged area of the vas deferens of the male reproductive system that serves as a storage reservoir for sperm.

Semiochemical A chemical that is involved in the communication between two organisms.

Semper cell A cell of the ommatidium that produces the crystalline cone.

Sensillum A sense organ.

Sensillum lymph The hemolymph that bathes the dendrite of a sensillum.

Sensory neuron The nerve cells that innervate sensory organs.

Sensory transduction The process of transforming environmental energy into biological energy.

Sex pheromone A chemical released from a gland of an individual of one species and causes members of the opposite sex of that species to orient toward it for mating.

Soma The cell body of a neuron.

Somatic mesoderm One of the mesodermal bands which develops during embryogenesis and gives rise to skeletal muscles, fat body, and a portion of the reproductive system.

Spermatheca A special sac in the female reproductive system that receives, stores, and releases sperm to fertilize the egg as it passes by in the oviduct.

Spermatid A male reproductive cell arising from the division of spermatocytes that contains the haploid chromosome number. The spermatid ultimately differentiates into a spermatozoan.

Spermatocytes A male reproductive cell that arises from the division of a male germ cell, the spermatogonium.

Spermatogonia The primoridial male germ cell.

Spermatophore A secretion of the male accessory glands that surrounds the sperm and protects it during transit to the female.

Spiracle The usually paired aperature in the integument that serves as the opening of the tracheal system to allow gaseous exchange.

Spiracular gill An extension of the cuticle that surrounds a spiracle, forming a gill that allows both aquatic and terrestrial respiration to occur.

Splanchic mesoderm One of the two mesodermal bands that develop during embryogenesis, giving rise to the visceral muscles.

Stomodeum The anterior epidermal invagination during embryogenesis that produces the foregut of the alimentary tract.

Storage excretion The ability to sequester metabolic wastes in the body.

Storage hexamerin One of a family of proteins that act primarily as storage proteins to provide amino acids required for protein synthesis in those developmental phases that do not feed.

Stylet sheath The salivary secretion of phytophagous hemipterans that seals the mouthparts around the plant surface.

Subesophageal ganglion The first ganglion of the ventral nerve cord, consisting of the fused ganglia of the mandibular, maxillary, and labial segments.

Subgenual organ A chordotonal organ usually located in the tibia, attached at one end to the cuticle and at the other to a trachea.

Superposition eye A variety of compound eye found in insects that maneuver under low light conditions. The movement of screening pigments allows light from neighboring ommatidia to stimulate other ommatidia.

Symbiont An organism that lives with an organism of another species.

Synapse The gap between two neurons, where electrical energy from nervous transmission is converted into chemical energy to stimulate the postsynaptic neuron.

Synchronous muscle A primitive wing muscle that requires nervous stimulation for each contraction.

Syngamy The fusion of male and female gametes to form a zygote.

Synomone A chemical produced by an individual of one species and received by another that has an adaptive effect on both.

Sytematics The study of classification and evolutionary relationships among taxa.

Tachykinin A peptide that stimulates the contraction of visceral muscles, homologous with the vertebrate peptides.

Taenidia The spiral thickenings of the tracheal epicuticle that prevent the collapse of the tracheal tubes from air pressure.

Taxis An oriented response to a stimulus.

Temporal polyethism The changes in behavior that accompany aging.

Testis The male reproductive organ that produces sperm, usually consisting of a pair of testes.

Thecogen cell An accessory cell that produces the sheath of the sensillum that isolates the axons from one another and provides them with nutrients.

Thelotoky Parthenogenetic reproduction resulting in only female progeny.

Tokus A hemolymph compartment at the tip of the abdomen containing tufts of aerating trachea that oxygenate hemocytes as they circulate through the hemocoel.

Tonic response The response of a sensillum that is strong when initially deformed and steady but reduced under constant deformation.

Tormogen cell The modified epidermal cell that produces the socket of a seta or sensillum.

Tracheal gills Evaginations of the integument that are covered by a thin cuticle and richly supplied with tracheae to allow aquatic respiration.

Tracheoblast An ectodermal cell that gives rise to a tracheole.

Tracheole A tracheal end cell that transfers oxygen from the tracheal system to body tissues.

Trail pheromone A volatile chemical that is laid down by foraging members of one species and is used by others of that species to locate the resources.

Transcription The process of copying a gene from the DNA to messenger RNA.

Transfer RNA A family of RNA molecules that specify certain amino acids that are to be brought to the ribosome for protein synthesis.

Translation The process of determining the amino acid sequence of a peptide from the sequence of nucleotides on messenger RNA.

Transverse tubule Invaginations of the sarcolemma to form an internal system of membranes that carries a depolarization deep within the muscle cell to the sarcoplasmic reticulum.

Trehalose A disaccharide that is the principal hemolymph sugar in insects.

Triacylglycerol Uncharged esters of glycerol that serve as the storage form of fatty acids.

Trichogen cell A modified epidermal cell that produces a seta.

Trophocyte A nurse cell that provides the developing oocyte with nutrients.

Tropomyosin A regulatory peptide complexed with the actin myofilaments.

Troponin A regulatory peptide complexed with the actin myofilaments.

Tunica propria The noncellular envelope that completely surrounds the ovariole.

Tymbal A sound-producing organ.

Tympanum An auditory organ consisting of a thinly stretched integumental membrane with a group of chordotonal sensilla below.

Urea A primary waste product of mammals, reptiles, and birds.

Uric acid The primary waste product of insects and some vertebrates.

Vas deferens The duct of the male reproductive system that connects the vas efferens with the ejaculatory duct. It may be enlarged into a seminal vesicle for the storage of sperm.

Vas efferens A tube that connects each testicular follicle with the vas deferens.

Ventral diaphragm An internal septum that divides the body cavity into com-

partments, located between the ventral nerve cord and the gut.

Ventral nerve cord The chain of interconnected ventral ganglia, connecting to the tritocerebrum by the circumesophageal connectives and extending to the end of the abdomen.

Visceral nervous system The portion of the nervous system that innervates and controls the gut and endocrine organs.

Vitellarium The area of the ovariole in which oocytes deposit yolk during vitellogenesis.

Vitellin The vitellogenins that have been deposited in the cytoplasm after modification by the follicular epithelium.

Vitelline envelope The membrane surrounding the yolk within an egg that forms the innermost layer of the chorion.

Vitellogenesis The process by which yolk produced by the fat body is taken up by the oocyte and deposited in its cytoplasm.

Vitellogenin A female-specific yolk protein that is synthesized by the fat body and taken up by the oocyte through receptor-mediated endocytosis.

Vitellophage An extraembryonic cell that digests the yolk stored within the egg that is used for embryogenesis.

Viviparity A method of reproduction in which a female gives birth to live offspring that have hatched within her body.

Wandering behavior A stereotyped set of behaviors that are initiated before pupation and place the last instar larva in a suitable environment to pupate.

Wax layer A lipid-containing layer of the epicuticle that serves as a major barrier to water loss.

Wild type The normal, most frequent form of an organism.

Z line The disc of protein within a muscle cell that separates sarcomeres.

Zinc finger protein A protein belonging to a protein family characterized by a highly conserved region of 30 amino acids that form fingerlike projections held together by zinc ions. The structure of the molecule enables the protein to bind to certain regions of the DNA molecule and regulate its transcription.

Zygote A fertilized egg resulting from the fusion of male and female gametes.

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